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THE EFFECT OF GRAPES AND GRAPE PRODUCTS ON URINARY ACIDITY

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Introduction

During the course of a study in this laboratory of the composition of California grapes and grape products, it was observed in all cases that the ash was decidedly alkaline, and it seemed likely that they might be of value in the diet for the purpose of maintaining the alkaline reserve. Since many fruit acids are largely oxidized to carbon dioxide and water in the body, the ash constituents rather than the organic acids are the chief factors affecting the acid-base balance of the body. However, some fruits have been shown to increase the acidity of the urine even though the ash was alkaline. Consequently, it has seemed desirable to secure additional information upon the effect of grapes and grape products upon the acidity of the urine.

The alkalinity or acidity of the ash of many food materials has been reported by Sherman and Gettler (15) who give a high alkalinity for the ash of raisins. Numerous published analyses of fresh grapes (2, 3, 14, 15) have indicated that a basic ash is characteristic of all varieties. Richert(13) has published results of his determinations of the alkalinity of the ash of several California (Vinifera) grape juice concentrates which indicate that the alkalinity is several times that of fresh grapes. Hartman and Tolman (8) have found the alkalinity of the ash of Concord or so-called Eastern grape juice to be relatively high.

Blatherwick (4) found that the addition of raisins to an otherwise acid diet resulted in decreased acidity of the urine. However, he did not state the source of the raisins nor did he report a study of fresh grapes and grape products. Therefore, it appeared desirable to ascertain whether the property of reducing the urinary acidity is characteristic of California varieties of grapes and their products. Pickens and Hetler (12) in testing the effect of a commercial Concord grape juice, found that "no marked change oc-

curred in the acidity of the urine, even when large amounts of grape juice were ingested." Their results and those of Blatherwick will be referred to later in this paper and a suggested explanation given for the results obtained by Pickens and Hetler.

Various periods have been employed for determining the body reaction equilibria both with a basal diet and with a basal diet plus the food material being studied. Blatherwick (4) used a five-day period for determining the effect of the basal diet. With regard to the influence of an added food on the composition of the urine, he stated that "the body reacts quickly to the change, and the new equilibria will generally have been established by the second day. Somewhat less than a week is sufficient to obtain conclusive data." Two subjects were used by them for study of the influence of each of several foods. Blatherwick and Long (5) in studying the effect of drinking large amounts of strained orange juice employed a four-day basal diet period followed immediately by a four-day period with daily increasing amounts of the juice added to the basal ration. Two subjects also were employed in these studies. In a corresponding study of the effect of sour milk, they employed two five-day periods with two subjects. Chaney and Blunt (6) studied the effect of orange juice on two growing children and in one case employed a preliminary period of three days during which no analyses were made. In the second case, the preliminary period was of five-days' duration with no analyses made. In both cases analyses were made during the following three days of basal diet, then after a delay of a day or two with basal plus orange juice, analyses were made for three days' continuation of the use of orange juice. Pickens and Hetler (12) studied the effect of grape juice on nitrogen retention and urinary acidity. Their experiments were divided into two periods. The first five days were for adjustment and usually no collections of urines or feces were made. A three- or four-day period followed in which daily urines were collected under toluene and analyzed for nitrogen. They reported their results as averages for the periods during which analyses were made. No data are given for any of the daily analyses. Two subjects were employed in each case. The results of Blatherwick (4), Blatherwick and Long (5), and Chaney and Blunt (6) indicate that equilibrium is obtained within five days on the basal diet. In like manner, it is shown that equilibrium was again attained within two or three days after the addition of the food to be studied.

Experimental

In this investigation the effect of fresh grapes and of several grape products on the reaction, ammonia content, total acidity and organic acid

content of the urine was determined. All samples were of known history. The fresh grapes were taken from a shipment of well ripened commercial Malaga grapes which appeared to be of uniform maturity and representative of the variety. The edible portion contained 23.6 per cent total solids. The natural grape juice was prepared by mixing one part of Malaga, one part of Muscat and one part of Petit Sirah freshly pressed grape juices, all containing 21 per cent total solids. The individual juices were portions taken from fifty to one hundred gallon quantities of juice prepared from mature, sound fruit. The detartrated grape juice was prepared with the same proportions of the individual juices but was detartrated by freezing (10). The natural grape juice concentration was prepared by mixing equal portions of the concentrated juices of the three varieties of grapes. The concentrate contained 72.3 per cent total solids. The concentrate-tartrate mixture consisted of one part concentrate and four parts of the tartrate precipitated from the juice by freezing. The Thompson seedless, and Muscat seeded raisins were from fifty-pound lots of the standard, commercially packed, dried fruit.

The basal diet was similar to that used by Chaney and Blunt (6), being as follows: whole milk, 275 gms.; oatmeal, 20 gms.; sugar, 10 gms.; white bread, 310 gms.; butter, 45 gms.; potato, 200 gms.; rice, 55 gms.; raw apple, about 150 gms.; egg, about 65 gms. This was followed in every case except in that of the feeding of the concentrate-tartrate mixture in which the diet included a constant added quantity of apple, lettuce and milk. Such a basal ration was chosen so that the urine would be considerably more acid than normal. This made it possible to secure a wide range of variation of the urinary pH without producing an alkaline urine and the possible consequent varying equilibria.

It seemed desirable to adopt a given length of time for an experiment and to report all daily analyses. It would thus be possible to study the daily as well as the final influence of the food upon the composition of the urine. A total time of ten or eleven days was therefore decided upon. Each experiment was divided into two periods. For the first five days each person received only the basal ration. During the next five (or six) days each person received, in addition, a constant quantity of fresh grapes or some grape product.

Whereas previous investigators have used two subjects only, in these experiments with fresh grapes, whole grape juice and detartrated grape juice, three subjects were used. Two subjects were given the grape juice concentrate and one was kept on a shortened test of concentrate-tartrate mixture. Two subjects were used with each kind of raisin, giving a total of

four persons on these two very similar products. It appeared that, with such a plan of study, the results in general would be more reliable than with a smaller number of subjects.

Since Blatherwick (4) had used a 300 gm. daily portion of raisins, it seemed desirable to employ this quantity in the present study so that the results in both should be comparable. Consequently, approximately equivalent quantities, on a solids basis, of fresh grapes, grape juice, and grape juice concentrate were used. The daily portions were as follows: 1000 gm. fresh grapes, 1000 cc. natural grape juice, 1000 cc. detartrated grape juice, 300 gm. natural grape juice concentrate, and 40 gm. of the concentrate-tartrate mixture. Altogether, twelve young men connected with the institution and carrying on approximately the same daily schedules of work, recreation and sleep volunteered their services.

All analytical determinations were made in duplicate or triplicate, the average of closely agreeing duplicates only, being reported. The following methods were employed: pH, according to the method of Palmer, Salvesen, and Jackson (11) and by the quinhydrone electrode (average of colorimetric and electrometric determinations reported, since agreement of pH = 0.05 was obtained); organic acids, Van Slyke and Palmer (17); ammonia, Van Slyke and Cullen (16); and titratable acidity, Henderson (9). Urine samples for 24-hour periods were collected, the experimental day ending just before breakfast each morning. The total volume was determined and 400 cc. specimens preserved with a few cc. of toluene. The pH determinations were made immediately, total acidity and organic acid determinations within twenty-four hours, and ammonia determinations within two or three days.

In addition to the urine analyses, determinations of the ash, the alkalinity of the soluble ash, and the alkalinity of the insoluble ash were made (1) on composite samples of the fresh grapes and on each of the grape products.

Results and Discussion

As is shown in Tables I to VI, the effect of the grapes and grape products on the pH of the urine is quite marked. On the basal diet the pH generally became stabilized at 5.85 to 5.70. It is interesting to note the different individual reactions shown in these experiments. For example, Subject C in Table I and Subject F in Table II remained at a pH of 6.0 on the basal ration; whereas several subjects showed a pH as low as 5.65. Since the data are continuous, it is possible to observe the nature of the daily changes occurring. It is apparent that this change is generally uniform with a tendency for pronounced increase of pH immediately following the inital in-

TABLE I

EFFECT OF FRESH GRAPES ON COMPOSITION OF URINE

Day*	Volume	pH	0.1-N Organic acids	0.1-N Titratable acids	Ammonia N	Volume	pH	0.1-N Organic acids	0.1-N Titratable acidity	Ammonia N	Volume	pH	0.1-N Organic acids	0.1-N Titratable acidity	Ammonia N
	Subject A					Subject B					Subject C				
	cc.		cc.	cc.	mg.	cc.		cc.	cc.	mg.	cc.		cc.	cc.	mg.
1	1100	5.85	590	116	55	735	5.65	474	160	54	990	6.50	337	127	89
2	1130	5.90	445	79	70	1110	5.80	468	175	81	1265	6.00	623	89	99
3	1215	6.15	572	78	88	950	6.00	401	84	59	610	6.00	459	55	99
4	1280	5.60	633	225	108	1390	5.60	643	234	101	840	6.20	547	145	216
5	1250	5.70	603	165	105	1870	5.65	755	176	115	670	6.00	385	99	180
6	1690	5.60	558	220	85	1460	5.60	546	196	57	1125	6.45	651	153	69
7	1520	6.30	645	37	51	1440	5.95	615	130	64	1430	6.75	560	75	66
8	1540	6.65	620	34	43	1300	6.25	702	125	65	780	6.55	615	49	48
9	1785	6.60	757	14	40	1900	6.60	836	45	63	980	6.65	558	33	44
10	1680	6.75	703	17	38	900	6.60	415	49	35	1760	6.80	587	60	39

* Days 1 to 5 inclusive, basal diet.
Days 6 to 10 inclusive, basal plus 1000 gms. fresh Malaga grapes.

TABLE II

EFFECT OF WHOLE GRAPE JUICE ON COMPOSITION OF URINE

Day*	Volume	pH	0.1-N Organic acids	0.1-N Titratable acidity	Ammonia N	Volume	pH	0.1-N Organic acids	0.1-N Titratable acidity	Ammonia N	Volume	pH	0.1-N Organic acids	0.1-N Titratable acidity	Ammonia N
	Subject D					Subject E					Subject F				
	cc.		cc.	cc.	mg.	cc.		cc.	cc.	mg.	cc.		cc.	cc.	mg.
1	890	6.05	475	93	90	2510	6.55	864	151	211	2060	5.90	667	140	173
2	1000	5.65	480	144	78	3605	5.90	577	144	202	2770	6.15	586	66	140
3	1200	5.80	484	150	88	2000	5.70	640	192	101	2960	6.25	698	71	133
4	820	5.80	436	100	78	1140	5.90	506	146	115	2100	5.90	680	164	129
5	940	5.85	489	98	99	1900	5.65	695	186	160	2460	6.00	674	118	138
6	970	5.90	654	169	109	1130	6.15	604	185	133	1930	6.60	710	97	97
7	1710	6.05	622	144	96	1010	6.05	540	208	91	1620	6.10	697	172	91
8	1750	6.50	514	99	59	4030	6.60	975	65	90	1860	6.10	614	138	83
9	1810	6.60	760	44	61	1650	6.60	828	33	56	1560	6.60	715	17	78
10	1720	6.65	760	21	67	2000	6.50	804	32	45	2150	6.50	855	47	72
11	1050	6.45	572	20	70	—	—	—	—	—	2660	6.65	783	53	74

* Days 1 to 5 inclusive, basal diet.
Days 6 to 10 inclusive, basal diet plus 1000 cc. whole grape juice.

TABLE III

EFFECT OF DETARTRATED GRAPE JUICE ON COMPOSITION OF URINE

Day*	Volume	pH	0.1-N Organic acids	0.1-N Titratable acidity	Ammonia N	Volume	pH	0.1-N Organic acids	0.1-N Titratable acidity	Ammonia N	Volume	pH	0.1-N Organic acids	0.1-N Titratable acidity	Ammonia N
	Subject D					Subject G					Subject H				
	cc.		cc.	cc.	mg.	cc.		cc.	cc.	mg.	cc.		cc.	cc.	mg.
1	900	6.45	526	78	81	830	6.40	474	32	65	1140	6.20	595	50	53
2	1025	6.05	574	33	69	790	5.80	588	128	117	1140	6.05	514	110	70
3	890	5.85	549	126	80	900	5.85	577	139	111	1610	6.00	622	106	81
4	860	5.85	484	122	96	960	5.80	533	167	140	1770	5.80	610	181	99
5	950	5.85	527	139	91	1170	5.85	592	157	131	1550	5.85	459	135	69
6	1860	6.05	596	112	94	1185	6.00	685	211	179	1520	5.95	572	110	85
7	1800	6.40	572	86	101	1630	6.40	640	95	91	1690	6.20	514	101	66
8	1620	6.45	649	84	63	2015	6.40	660	52	102	1830	6.25	610	77	102
9	1100	6.20	495	90	71	1680	6.40	655	81	118	2100	6.40	651	50	71
10	720	6.00	636	76	68	1180	6.00	560	135	96	1940	6.40	637	43	74
11	710	6.25	530	14	20	1730	6.40	643	138	85	—	—	—	—	—

* Days 1 to 5 inclusive, basal diet.
Days 6 to 10 inclusive, basal diet plus 1000 cc. detartrated juice.

TABLE IV

EFFECT OF CONCENTRATE AND CONCENTRATE-TARTRATE MIXTURE ON COMPOSITION OF URINE

Day*	Volume	pH	0.1-N Organic acids	0.1-N Titratable acidity	Ammonia N	Volume	pH	0.1-N Organic acids	0.1-N Titratable acidity	Ammonia N	Volume	pH	0.1-N Organic acids	0.1-N Titratable acidity	Ammonia N
	Subject I (Concentrate)					Subject D (Concentrate)					Subject F (Concentrate-Tartrate)				
	cc.		cc.	cc.	mg.	cc.		cc.	cc.	mg.	cc.		cc.	cc.	mg.
1	815	6.10	531	82	123	775	6.15	413	54	83	—	—	—	—	—
2	1250	5.85	520	95	118	820	5.85	611	126	98	2130	6.45	647	102	119
3	1380	5.90	607	61	77	1390	6.10	600	111	78	1940	6.60	655	39	87
4	1170	5.80	503	138	121	890	6.10	526	99	80	1020	6.40	600	50	49
5	1730	5.80	575	62	145	690	5.60	500	129	70	1500	6.40	639	61	51
6	1640	6.25	846	49	119	1270	6.05	465	127	85	2340	6.90	842	56	53
7	1760	6.45	598	85	109	960	6.30	588	48	59	1700	7.15	1280	88	28
8	1620	6.40	954	62	100	1100	6.55	714	35	49	1750	7.20	1021	91	20
9	1410	6.65	825	61	71	1760	6.80	587	60	39	—	—	—	—	—
10	1490	6.60	677	60	83	1050	6.80	624	42	47	—	—	—	—	—
11	2290	6.65	784	51	64	—	—	—	—	—	—	—	—	—	—

* Days 1 to 5 inclusive, basal diet.
Days 6 to 10 inclusive, basal diet plus 300 gm. concentrate.
Days 6 to 8 inclusive, basal diet plus 40 gm. concentrate-tartrate mixture.

gestion of the grapes or grape product. In all cases there is a distinct total increase in the value of the pH, whether the pH of the initial period was, for example, 5.9 or 5.6.

In general, this increase was such that the pH on the last day with the basal ration plus grape product was 0.8 to 1.0 pH unit higher than that of the last day with only the basal ration. In these experiments, this was true for fresh grapes, natural fresh grape juice, untreated fresh grape juice con-

TABLE V

EFFECT OF THOMPSON SEEDLESS RAISINS ON COMPOSITION OF URINE

Day*	Volume	pH	0.1-N Organic acids	0.1-N Titratable acidity	Ammonia N	Volume	pH	0.1-N Organic acids	0.1-N Titratable acidity	Ammonia N
	Subject J					Subject B				
	cc.		cc.	cc.	mg.	cc.		cc.	cc.	mg.
1	910	6.60	499	117	104	1050	5.95	391	177	73
2	980	5.85	499	110	93	1380	5.65	762	100	116
3	1125	5.70	550	158	76	1050	5.65	517	155	71
4	1230	5.70	542	187	89	1020	5.55	505	149	57
5	1140	5.65	570	194	115	1410	5.70	561	105	79
6	1070	5.75	665	178	126	1820	5.85	695	160	82
7	840	5.85	753	192	99	1270	6.45	707	95	93
8	1000	6.15	602	142	95	1020	6.20	564	78	80
9	1120	6.45	765	40	56	1170	6.40	794	65	53
10	1240	6.40	905	47	58	1220	6.50	856	62	48
11	1190	6.40	800	67	38	1430	6.30	616	60	56

* Days 1 to 5 inclusive, basal diet.
Days 6 to 11 inclusive, basal diet plus 300 gm. raisins.

centrate, and for Thompson seedless and Muscat seeded raisins. As indicated in Table VI, even 100 grams of Muscat seeded raisins are effective in decreasing the reaction of the urine.

With the detartrated grape juice, the average increase of pH was only 0.5 to 0.6 units, or about one-half that produced by the untreated fresh juice, concentrate and raisins. With the concentrate-tartrate mixture the pH increase was approximately as great as with the natural juice, concentrate and raisins but the large quantity of added tartrate in the mixture rendered it unpalatable and the study was not continued after the third day. Even in this experiment, the excess of base in the grape product is

sufficient to balance the organic acidity and to reduce greatly the acid reaction of the urine.

From the data in Tables I to VI showing the values for total acids and ammonia excreted daily, it is apparent that there was a marked decrease in these factors when the grapes or grape products were added to the basal diet. In the experiment with the concentrate-tartrate mixture the effect was not so decided although with even the excessive amounts of tartrate

TABLE VI

EFFECT OF MUSCAT SEEDED RAISINS ON COMPOSITION OF URINE

Day*	Volume	pH	0.1-N Organic acids	0.1-N Titratable acidity	Ammonia N	Volume	pH	0.1-N Organic acids	0.1-N Titratable acidity	Ammonia N
	Subject A					Subject H				
	cc.		cc.	cc.	mg.	cc.		cc.	cc.	mg.
1	870	6.70	592	174	59	1420	6.30	579	80	96
2	860	5.80	509	114	77	1920	5.70	661	146	129
3	1480	5.80	474	161	86	920	5.65	486	184	93
4	1820	5.80	641	197	102	1240	5.65	536	213	97
5	1020	5.75	443	150	96	1180	5.75	560	196	124
6	1520	6.15	995	128	102	1110	5.80	688	158	100
7	820	6.05	563	120	64	1470	6.25	443	62	91
8	830	6.00	990	113	56	1040	6.25	604	95	47
9	950	6.30	820	72	43	1150	6.50	816	55	39
10	950	6.85	650	27	32	1240	6.50	832	47	55
11	980	6.70	611	8	27	1130	6.55	650	45	44

* Days 1 to 5 inclusive, basal diet.
Days 6 to 9 inclusive, basal diet plus 300 gms. raisins.
Days 10 to 11 inclusive, basal diet plus 400 gms. raisins.

ingested, the total acid increase is only equal to the drop in the ammonia excreted.

A further significance of these results is obtained from a consideration of the relationship between the alkaline reserve and the acid and ammonia excretions. Fitz and Van Slyke (7) have shown that in normal men "the excretion of acid in excess of fixed bases as measured by determining the ammonia and titratable acid bears a quantitative relationship to the alkaline reserve of the body as measured by the CO_2 binding power of the blood plasma." They have developed an evaluation of this relationship which is

$$\sqrt{D/W\sqrt{C}}$$

where D represents the rate of excretion of 0.1 N ammonia plus titratable acid per 24-hour time unit, C the 0.1 N NH_3 plus acid per liter of urine and W the body weight. They assume the acid accumulation in the plasma is proportional to the fall of the plasma CO_2 figure below the maximum plasma CO_2 capacity expressed in volumes per cent. This fall of the plasma CO_2 is then given by the expression above. Consequently, as $\sqrt{D/W\sqrt{C}}$ increases, there is a corresponding decrease in the plasma CO_2 and an indication of the change of the plasma CO_2 level is obtained. This affords an estimate of the decrease of the alkaline reserve. In their studies with normal individuals, the value of the alkaline reserve as determined in the urine usually had an average error of 3.2 volume per cent.

Using the values for titratable acid, ammonia, and total volume given in Tables I to VI and the curves of Fitz and Van Slyke (7), the values for the index $\sqrt{D/W\sqrt{C}}$ were computed. These are given in Table VII. Within the limits of accuracy, it is apparent that the increase of the index was general in all the basal diet periods. Correspondingly, there was a marked decrease in the index when the grapes or grape products were added to the basal diet. In most cases the grapes or grape products decreased the index by one-half or more. This decrease is an average index of 2.8 volumes per cent below the maximum plasma CO_2 capacity. If this maximum is taken as 80 volumes per cent (7) and the normal value for all experiments as 80 volumes per cent minus the average index for the first day, or 74.7 volumes per cent, it is evident that with a final average value of 77.2 volumes per cent the plasma CO_2 capacity has been increased above the normal by the grapes and grape products. The exception was that of Subject G with the detartrated juice in which case the total decrease of the index was nevertheless very marked. It may therefore be concluded that grapes and grape products such as were employed in these experiments, assist in securing a high alkaline reserve as estimated by the plasma CO_2 binding power determined from the acid and ammonia content of the urine.

A contrast to the results obtained in these experiments is presented by those of Pickens and Hetler (12) who have reported that no marked change occurred in the acidity of the urine. Their published data are very condensed, neither the results of the daily analyses nor the values of the urinary ammonia and total volumes being given. It is, therefore, impossible to observe either the general daily trend or to calculate any other data from their results.

It was known that the California grapes and grape products which are here shown to be markedly basic in physiological reaction also yielded an

TABLE VII

INDEX OF LOWERING OF PLASMA CO_2 CAPACITY
(Volumes Per Cent)

Day	Fresh grapes	Fresh grapes	Fresh grapes	Whole grape juice	Whole grape juice	Whole grape juice	Detartrated grape juice	Detartrated grape juice	Detartrated grape juice	Grape juice concentrate	Grape juice concentrate	Concentrate-tartrate	Thompson seedless raisins	Thompson seedless raisins	Muscat seeded raisins	Muscat seeded raisins
	A	B	C	D	E	F	D	G	H	I	D	F	J	B	A	H
1	5.0	6.3	5.4	5.0	6.1	6.8	4.9	3.1	3.0	6.4	4.5	—	6.6	6.5	6.9	4.3
2	4.8	6.3	4.9	6.1	6.0	4.2	3.5	6.8	4.9	5.7	6.5	5.1	5.6	5.0	5.9	5.6
3	4.9	4.1	4.9	6.0	5.7	4.0	6.0	6.5	4.4	4.4	5.2	3.3	5.9	6.0	6.1	6.7
4	8.0	7.3	8.1	5.8	6.0	6.0	6.1	7.2	6.0	7.5	5.3	3.4	6.4	5.8	6.8	7.3
5	7.0	6.2	5.0	5.4	6.6	5.1	6.4	7.1	5.0	5.2	7.0	3.3	7.4	5.0	6.6	7.1
6	7.0	6.0	5.5	8.4	7.0	4.5	5.0	8.1	4.9	5.1	5.8	2.7	7.4	5.5	5.8	6.1
7	3.0	5.0	3.9	5.5	7.0	6.5	4.7	4.6	4.0	5.0	4.3	3.1	8.0	4.9	5.6	3.8
8	2.0	4.9	3.1	4.9	2.5	5.5	4.3	4.0	4.5	4.7	3.0	3.1	6.6	4.5	5.1	3.9
9	1.6	2.9	3.0	3.0	2.1	3.0	4.5	4.7	3.0	4.2	3.0	—	3.0	3.2	4.0	3.1
10	1.5	2.8	3.0	2.5	2.0	3.0	4.8	6.0	2.9	4.3	2.9	—	3.1	3.1	2.1	3.1
11	—	—	—	2.5	—	3.7	1.8	5.5	—	3.6	—	—	3.1	3.1	1.1	2.8

ash that was highly alkaline. It therefore seemed desirable to learn whether there was any correlation between the alkalinity of the ash of the juice or grape product and the body reaction. Hartman and Tolman (8) have published the results of a comprehensive study of the chemical effects of the manufacture of Concord grape juice. Their studies were made in the districts in which the great bulk of such commercial juice is manufactured. Consequently their results are authentic and satisfactory for the purpose of comparison. Their averages of a great many determinations are taken to approximate the values for the commerical Concord juice employed by Pickens and Hetler. Their average results are given in Table VIII, to-

TABLE VIII

CHEMICAL COMPOSITION OF GRAPES AND GRAPE PRODUCTS

Materials	Ash per 100 gm.	Alkalinity soluble ash, N/10 Acid, per 100 gm.	Alkalinity insoluble ash, N/10 Acid, per 100 gm.	Total alkalinity N/10 Acid, per 100 gm.
	gms.	cc.	cc.	
Concord grape juice—at time of storing....	0.39	41.1*	7.5*	48.6*
Concord grape juice—after 4 month's storage	0.27	28.3*	4.0*	32.3*
Concord grape juice—after 16 month's storage	0.24	21.8*	5.9*	27.7*
Malaga grapes—fresh.....	0.60	77.1	31.2	108.3
Grape juice—fresh, used in diet.....	0.40	39.0	35.2	74.2
Grape juice—detartrated, used in diet.....	0.27	20.8	23.0	43.8
Grape juice concentrate, used in diet.....	1.33	152.0	94.5	246.5
Concentrate-tartrate mixture, used in diet..	18.4	2430.0	770.0	3200.0
Thompson raisins, used in diet.....	2.20	218.0	121.0	339.0
Muscat raisins, used in diet.....	2.07	234.0	106.0	340.0

* cc. N/10 Acid, per 100 cc. juice.

gether with those of grapes and the various grape products used in the present study. It will be noted that the values for the alkalinities of the Concord juices are for 100 cc. quantities while all others are for 100 grams. The relative differences are probably small in this case. The total alkalinity of the Concord grape juice decreases about 33 per cent during the first four months' storage and about 43 per cent during the entire first sixteen months' storage, resulting in a final average alkalinity of 27.7 cc. 0.1 N acid per 100 cc.

In sharp contrast to this alkalinity is that of the juice used in the present experiment of which the alkalinity is approximately 74 cc. 0.1 N acid per 100 gram. The detartrating of this fresh juice resulted in a decrease of the

total alkalinity of about 41 per cent of the original to a value of about 44 cc. 0.1 N acid per 100 grams. The decrease in total alkalinity of both juices is approximately the same for the two methods. However, the final total alkalinity of the detartrated juice used in the present diets is still nearly as high as that of the fresh Concord juice at the time of storing and before it was detartrated. The juices being compared then have the following approximate alkalinities as cc. 0.1 N acid per 100 grams: fresh grape juice used in the present experiment, 74; detartrated grape juice similarly used, 44; and 28 for the Concord grape juice as prepared for commercial use.

If the magnitude of the basic reaction of the body parallels that of the quantity of basic food ingested only in addition to a certain fixed basal ration, this basic reaction of the body is a consequence of the basic food so ingested. It has been shown here that with every experiment with fresh grape juice, as well as with grapes and certain grape products, the reaction of the body has become markedly basic in comparison to the normal reaction estimated from the analyses of the first day and before new equilibria had been effected. It is also evident that the detartrated grape juice used in these experiments and having somewhat more than one-half as basic an ash as the fresh juice, did not produce such a marked basic reaction, but that there was, nevertheless, a distinct basic effect. In fact, the average basic effect of the detartrated grape juice used here appeared to be approximately one-half that of the fresh grape juice and grape products of equivalent basic ash content. Further, it has been shown (12) that a Concord product with somewhat more than one-third the basic ash content (8) did not produce a marked basic reaction of the body. Since such a parallelism does exist, there is evidently a definite relationship between the body reaction and the alkalinity of the ash of grapes and grape products. The results here reported demonstrate that this is true for grapes, fresh grape juice, detartrated grape juice, fresh grape juice concentrate, Thompson seedless and Muscat seeded raisins of the types investigated.

From such a study it is evident that the divergency of the results of Blatherwick with raisins and those of Pickens and Hetler with a commercial Concord grape juice should be expected. This is because of the very marked difference in their respective basic properties as indicated by the alkalinity of the ash.

A study of the quantitative relationship between the alkalinity of the ash and the body reaction should be fruitful. Such a study will be reported in another paper from this laboratory.

From Tables I to VI inclusive, it is evident that there is an increased excretion of organic acids coincident with the decrease of both the total

TABLE IX

ORGANIC ACIDS INGESTED AND OXIDIZED

Subject	Fresh grapes			Whole grape juice			Detartrated grape juice			Grape juice concentrate		Concentrate-tartrate	Thompson seedless raisins		Muscat seeded raisins			
⟶	A	B	C	D	E	F	D	G	H	I	D	F	J	B	A		H	
Av. daily organic acid titration for basal diet period (cc. N/10 HCl)	565	548	470	473	656	661	532	553	560	547	530	635	532	547	300 gm. 532	400 gm. 532	300 gm. 564	400 gm. 564
Av. daily organic acid titration for period with basal diet+grapes, etc.	657	623	594	647	750	729	580	639	597	781	596	1047	748	705	771	630	672	741
Difference (cc. N/10 HCl)	93	75	124	164	94	68	48	86	37	234	66	412	216	158	239	98	108	177
Organic acids not oxidized (gm.)	1.02	0.85	1.34	1.68	1.05	0.76	0.53	0.96	0.41	2.50	0.71	4.58	2.41	1.76	2.66	1.09	1.20	1.97
Organic acids ingested daily (gm.)	27.8	27.8	27.8	22.2	22.2	22.2	12.7	12.7	12.7	31.4	31.4	36.1	24.1	24.1	23.7	31.7	23.7	31.7
Per cent oxidized	96.3	97.0	95.0	92.4	95.3	96.6	95.8	92.4	96.7	92.0	97.7	87.3	90.0	92.7	88.8	96.6	94.9	93.8

acidity and ammonia. This is similar to the effect of orange juice (5). This was explained as being probably the result of a part of the citric acid escaping oxidation in the body and thus being present in the urine and increasing the titration value for organic acids. The same explanation may apply in the case of the organic acids in grapes and grape products.

The organic acid content of the grapes and grape products was determined by the method of Van Slyke and Palmer (17). The total amounts daily ingested in each experiment are given in Table IX. The differences of the average daily organic acid titration of the urine with and without the grapes or grape products added to the basal diet are also given. From these values, the percentage of the organic acids oxidized in the body may be computed.

For example, the data with the fresh grapes may be considered. The Van Slyke and Palmer titration indicated that the organic acid content was equivalent to a concentration of 1.88 per cent as tartaric acid. Since by this method tartaric acid is titrated to the extent of about 67 per cent, the true value for total tartaric acid would be about 2.8 per cent. Therefore, approximately 28 grams would be taken daily by an individual. The average daily increase of excreted organic acids in the urine of the last five days above that of the first five, for Subject A with fresh grapes, was equivalent to 92 cc. of 0.1 N acid. Assuming that this increase was entirely due to tartaric acid, such acid in the urine would be about 1.02 grams. Therefore, approximately 3.7 per cent of the ingested tartaric acid of the fresh grapes appears to have escaped oxidation.

In this manner the total amount of organic acids ingested, the amount excreted, and the per cent organic acids oxidized, have been calculated and presented in Table IX. In each experiment with fresh grapes, whole grape juice, detartrated grape juice, whole grape juice concentrate, Thompson seedless and Muscat seeded raisins, the individual ability to oxidize the ingested organic acids is high, varying from approximately 90 to 97 per cent. An average of the sixteen experiments with varying quantities of total ingested tartrate indicates that approximately 94.4 per cent of the organic acid, as tartaric, is oxidized. For the concentrate(−)tartrate mixture with a very excessive amount of tartrate present, over 87 per cent of the tartrate, as tartaric acid, is oxidized. The average with all experiments of approximately 94 per cent oxidation compares quite interestingly with that of Blatherwick and Long (5) who reported approximately 94 per cent oxidation of citrates or organic acids, as citric acid, in experiments with orange juice.

SUMMARY

Sixteen experiments with men subjects on basal diet and on the same basal supplemented by fresh grapes, fresh natural grape juice, detartrated grape juice, natural grape juice concentrate, concentrate-tartrate mixture, Thompson seedless and Muscat seeded raisins are reported. The following results were observed when the grapes or grape products were added to the basal ration.

1. An increase in the pH of the urine occurred, ranging from 0.8 to 1.2 pH units.

2. A decrease in the ammonia excreted and a corresponding decrease in the total acidity was noted.

3. There was an increase of the alkaline reserve above the normal for each subject, the alkaline reserve being estimated by the plasma CO_2 combining power determined from the acid and ammonia content of the urine and calculated by the method of Fitz and Van Slyke.

4. A correlation between the alkalinity of the ash and the physiological reaction was apparent. Grapes and grape products with the greater alkalinity of ash were associated with a more basic body reaction.

5. An increase occurred in organic acids excreted. This may be explained by the presence in the urine of some incompletely oxidized tartaric acid.

6. Approximately 94 per cent of the ingested organic acids appeared to be oxidized.

These results are applicable to grapes and grape products of the varieties and types investigated.

The writer wishes to express his appreciation of the interest and thoughtful consideration of Professor W. V. Cruess, upon whose suggestion this study was initiated. The friendly advice of P. F. Nichols of this laboratory has been of great assistance. The full cooperation of the men taking a part in the diets is acknowledged with pleasure.

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A STUDY OF THE CALCIUM RETENTION ON A DIET CONTAINING AMERICAN CHEDDAR CHEESE

By

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COW'S milk has been generally recognized as a good source of calcium in the diet. Unfortunately, individuals often dislike milk, and since the commonly used American Cheddar cheese is made from cow's milk, it would seem of value to determine whether its calcium is as well utilized as the calcium of milk. From a survey of literature the experiments reported on the digestibility of cheese (1, 2, 3, 4) seem to indicate that cheese is well utilized. However, there do not appear to be any experiments reported on the calcium retention of American Cheddar cheese or of any other variety of cheese.

Cheese made by different methods, that is, with rennet and with lactic acid, contains different percentages of calcium as reported by Blunt and Sumner (5). They found the average per cent of calcium in American Cheddar cheese to be 0.71 per cent; Swiss cheese 1.05 per cent; while that in cottage cheese was 0.077 per cent. The calcium content of the American Cheddar cheese used in this calcium retention study was found to be of similar percentage namely, 0.74 per cent.

A compilation of the data of four investigators (6, 7, 8, 9) on the retention by adults of calcium of pasteurized whole milk is given in Table I.

Two healthy young women, one an assistant in Nutrition in the School of Home Economics and the other a graduate student in Nutrition, served as the subjects. They were comparatively similar in weight, namely, 51.3 kilograms and 50.2 kilograms. They were well within the correct range of weight for their height, had lived on an adequate diet previous to this study, and lived healthy, active lives. During this study the subjects were engaged in the same activities and were not exposed to direct sunlight.

The studies extended over a period of 18 consecutive days during February. The 18 days were divided into two periods of 9 days each, one for the study of American Cheddar cheese and the other for the study on pasteurized milk. Each 9-day period consisted of a 3-day preliminary period followed by a 6-day experimental period. During the preliminary period the same weighed amounts and kinds of food were eaten as those of the experimental period.

The diets were planned to furnish an amount of calcium that would be close to the calculated average requirement for equilibrium (10) and to meet the protein and energy requirements of the subjects. The diet for the study of the calcium retention of cheese consisted of American Cheddar cheese, lean round beef, cored apple, soda crackers, butter, and sucrose. In the milk study the diet was the same except that pasteurized milk replaced the American Cheddar cheese. Distilled water was used *ad libitum* for drinking.

TABLE I

COMPILATION OF CALCIUM STUDIES ON PASTEURIZED MILK

Investigator	Subjects adult women	Body weight	Calcium from milk	Intake per kg. per day	Calcium balance per day	Calcium balance per kg. per day
		kg.	per cent	mg.	gm.	mg.
Rose* (1920)	E. B.	54	70	7.1	+.060	+1.1
	R. E.	56	70	6.8	+.087	+1.5
Rose and MacLeod* (1923)	B. B.	54	70	6.0	−.024	−0.4
	G. C.	62.5	74	6.4	+.008	+0.1
	C. M.	45	71	6.6	−.039	−0.8
Willard and Blunt (1927)	3 adults—1 serving twice; making 4 comparisons	no weights given	50	no data	−.09 −.16 −.01 −.03	no data
Kramer, Latzke, Shaw (1928)	E. L.	53.6	68	9.4	−.242	−4.5
	I. B.	58.2		8.6	−.122	−2.1
	A. L.	68		7.4	−.125	−1.8
	M. K.	56.3		8.9	−.098	−1.7

* Personal communication.

The American Cheddar cheese[1] was made in September, 1930. "The milk from which the cheese had been made was pasteurized at 160°F and was not held at this temperature for any length of time." The vitamin A content of the same American Cheddar cheese as used in this study was tested in the Nutrition Laboratory and it was shown that 0.5 gram per day was as effective as 0.8 and 1.0 gram in the prevention of eye disease and in the production of normal growth in albino rats. Sherman's (11) quantitative method was used.

[1] Obtained through the courtesy of Professor H. W. Gregory, Department of Dairy Husbandry, Purdue University, from the Kraft Phenix Cheese Company at Marion, Indiana.

The milk, consumed as purchased, was obtained fresh each day from the Purdue University Creamery. It was milk that had been pasteurized at 142° to 145°F, held at that temperature for at least 30 minutes, then cooled immediately to 50°F or less.

The calcium from the cheese furnished 86.0 per cent of the total calcium of the diet for one subject and 85.7 per cent of the total calcium for the other. The calcium from the milk furnished 87.7 per cen of the total calcium of the diet for one subjec and 87.4 per cent for the other.

A sufficient amount of food, with the exception of milk, butter, and meat, was purchased at one time for both experiments. The meat was lean round of beef purchased from the local market in two lots. It was trimmed of fat, ground, and well mixed. Samples were taken for calcium analysis. A day's supply for each subject was weighed for the entire experimental period, wrapped in oil paper, and kept in cold storage. The meat was baked in casseroles in a hot oven from 10 to 20 minutes, according to the preference of the subject. It was eaten from the same container in which it was baked in order to avoid loss. The apples, of the winesap variety, chosen for flavor, were purchased from the local market and were kept in cold storage. To meet the caloric requirement, sugar was eaten as pure cane sugar made into a flavored paste. The daily supply of sucrose was evenly divided for the three meals. The protein of the diet was of a high biological value and was consumed in the amount of at least one gram per kilogram of body weight. The Torsion balance used for the weighing of the food consumed had its weights checked to the second decimal place with quantitative weights.

The daily intake of calcium is given in Table II. In the cheese study, the average daily calcium intake for each subject was 10.2 milligrams per kilogram of body weight; and in the milk study, 9.4 milligrams per kilogram. These amounts were sufficient for calcium equilibrium for women subjects as indicated by the work of other investigators (9, 12, 13, 14, 15).

For the calcium determinations weighed samples of American Cheddar cheese, pasteurized milk (a composite sample for every three days), cored apple, lean ground beef, and soda crackers were dried in an electric oven and then ashed in porcelain crucibles according to the official method (16) using an electric muffle furnace, kept between 400°C and 450°C.

Urine and feces of the 3-day preliminary period were not collected. The first collection of the excreta was made the first day of the experimental period. The feces were dried and ashed for calcium analysis.

Calcium of food, feces, and urine was determined by McCrudden's method (17) with the *pH* value adjusted according to Shohl and Pedley

(18). The 0.01N and 0.05 N $KMnO_4$ solutions were prepared and standardized according to Halverson and Bergeim (19). The potassium permanganate solution used in any series of calcium determinations was restandardized before the titrations for calcium were made. For titrations U. S. Bureau of Standard burettes were used. Redistilled water was used for the preparation of all solutions and for rinsing of all apparatus used in making

TABLE II
DAILY FOOD INTAKE

Food	American Cheese Experimental Period				Pasteurized Milk Experimental Period			
	Subject I (51.3 kg.)		Subject II (50.2 kg.)		Subject I (51.3 kg.)		Subject II (50.2 kg.)	
	Wt.	Calcium	Wt.	Calcium	Wt.	Calcium	Wt.	Calcium
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Cheese, Amer. Cheddar	61	.450	59.5	.439	—	—	—	—
Milk, whole pasteurized	—	—	—	—	338	.421	330	.411
Apples, fresh, cored but unpared	300	.014	300	.014	300	.014	300	.014
Beef, lean round	150	.022	150	.022	150	.008	150	.008
Crackers, soda	75	.031	75	.031	75	.031	75	.031
Butter	40	.006*	40	.006*	40	.006*	40	.006*
Sucrose	200	—	180	—	200	—	180	—
Total		.523		.512		.480		.470

* Rose, M. S., Laboratory Handbook for Dietetics, 3rd edition 1929.

the analyses. The chemicals used were secured from the Mallinckrodt Chemical Works. The modified calcium method was subjected to preliminary testing for the recovery of calcium from known solutions. Determinations of calcium were made in triplicate.

In Table III are given the average daily calcium intake and output for the subjects for both the American Cheddar cheese study and the pasteurized milk study.

Discussion and Results

The calcium balances, as given in Table III, show a daily average balance of +0.005 grams for Subject I and a daily average balance of −0.040 grams for Subject II on the diet in which American Cheddar cheese was used; and the calcium balances show a daily average balance of −0.010 grams for Subject I and a daily average balance of −0.031 grams for Subject II on the diet in which pasteurized whole milk was used.

TABLE III

CALCIUM BALANCE (6 DAYS)

Subject	Experimental period	Calcium Intake		Calcium Output			Balance	Balance per day	Balance per kg. per day
		total	per kg. body weight	urine	feces	total			
		gm.	mg.	gm.	gm.	gm.	gm.	gm.	mg.
Subject I weight 51.3 kg.	Cheese, Amer. Cheddar	3.138	10.2	1.099	2.012	3.111	+0.027	+0.005	+0.1
	Milk, whole pasteurized	2.871	9.4	0.590	2.343	2.933	−0.062	−0.010	−0.2
Subject II weight 50.2 kg.	Cheese, Amer. Cheddar	3.072	10.2	0.538	2.774	3.312	−0.240	−0.040	−0.8
	Milk, whole pasteurized	2.811	9.4	0.484	2.524	3.008	−0.187	−0.031	−0.6

Comparing the calcium balances of Subject I on the two diets, the results indicate that Subject I changed from an almost negligible positive balance to a slight negative balance; i.e., Subject I remained almost in calcium equilibrium. On both the cheese and pasteurized milk diets, Subject II showed slightly negative balances of approximately equal value.

It would seem, therefore, if Subject I were almost in calcium equilibrium and Subject II showed negative calcium balances of approximately the same value, that the calcium of the cheese was as well utilized as the calcium of the milk.

During one day of the cheese experimental period Subject II had four bowel movements instead of the usual one. The two young women subjects maintained their weight and remained in good health throughout the experiment.

The results of this experiment, in which two healthy young women served as subjects, seem to indicate that the calcium of the American Cheddar cheese was as well utilized as the calcium from the pasteurized whole milk.

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A QUANTITATIVE STUDY OF THE DIETARY OF THE HUMAN MOTHER WITH RESPECT TO THE NUTRIENTS SECRETED INTO BREAST MILK*

By

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THE present paper reports an investigation of the self-chosen diets and the nutritive requirements of three multiparae during 60 weeks of intensive lactation, with special reference to the intake of calories, proteins, fats, carbohydrates, calcium, and phosphorus in the food and the outgo of these substances in the milk secreted. These women produced an average of 1419 cc., 2366, and 3134 cc. of milk daily. In view of the current empiric regimens recommended for lactating women, it is pertinent to consider the quantity of nutrients actually utilized in the secretion of milk.

The principal organic constituents of milk, a secretion with a fairly constant chemical composition for each species, are casein and lactalbumin, milk fat, and lactose. These constituents, peculiar to milk, are secreted by the mammary glands from precursors such as amino acids, phosphatids, and glucose respectively, in the blood. All the organic and inorganic constituents of milk may be derived either from ingested food or from body tissue.

Though inorganic elements, vitamins, and some drugs and other substances appear to be transferred directly to milk, food proteins, fats, and sugars do not ordinarily follow this course (1 to 11).

Nevertheless, the woman who is producing an adequate amount of milk of good quality and doing so without any marked loss in body weight, must be transforming ingested food into milk. Meigs and Cary (12, p. 494) state:

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* A preliminary report of this investigation was presented before the Division of Biological Chemistry at the Eighty-First Meeting of the American Chemical Society in Indianapolis, March 31, 1931.

† National Research Council Fellow in Child Development and Parent Training under a grant of the Spelman Fund, 1927–28; National Research Council Fellow in Child Development under a grant of the Spelman Fund, 1929–30.

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that

> There is every reason to think, therefore, that, under ordinary circumstances, milk protein is made from food protein, milk fat from food fat, and milk carbohydrate from food carbohydrate. But it has also been clearly shown that this is not always the case, and the numerous other possible ways in which the mammary gland may get materials for the manufacture of its product must be kept clearly in mind in order to interpret the results of the numerous experiments which have been carried out to determine the effects of changes in the food supply on milk secretion.

It is known that some constituents are interconvertible in metabolism, and, this being so, milk fat and carbohydrate are not necessarily to be traced directly to the corresponding food substances. It has been demonstrated also that proteins may be transformed into glucose and glucose into fat (13, p. 267). Proteins, fats, and carbohydrates have been shown to serve as the initial source of milk fat in the lactating organism (14, 15). Since the mammary glands must compete with other organs for nutrients from the blood stream during the metabolic processes, there is a variation in the quantity and proportion of the various components available at any given time for milk synthesis. This variation is influenced by digestion, absorption, and environmental factors.

When the necessary ingredients for milk synthesis are lacking in the daily food, the mother may either decrease her milk output, secrete milk deficient in these constituents, or draw them from her own tissues. A marked deficiency of dietary proteins, fats, or carbohydrates is usually associated with a prompt drop in milk volume; inadequate vitamin intake may result in a vitamin-deficient milk; while an insufficient mineral intake results in a drain on the maternal reserves. Although intensive lactation is often marked by negative calcium and phosphorus balances (16, 17) there is obviously a limit to the amount of material a woman can lose from her tissues without some impairment of her body and a diminution in the nutritive value of her milk. The interrelations of these many dietary and metabolic factors in milk secretion and their application to the nutrition of the mother and child have been reviewed in detail elsewhere (12, 18, 19).

Experimental work to demonstrate the precursors of milk constituents and the factors determing the metabolic requirements for lactation, has been done chiefly on mammals other than man and especially on the cow and goat. Though the findings on these animals are of significance in indicating the general relationships, they cannot be expected to show the specific demands of woman. The digestive processes and the synthetic capacity of different species may vary (20). Investigations leading to a full knowledge of the processes of and requirements for lactation in woman will serve to replace our present empiric regimen for the lactating mother with a rational one. The present study may add apposite information in this direction.

Procedure

The subjects of this study were housewives carrying on their usual home duties. They had sustained continuous reproductive processes for a period of four to six years. Their diets were entirely self-chosen and consisted of those foods which they liked and which they believed to favor milk flow. The quantity of each food stuff ingested over intervals of 3 to 10 consecutive days was weighed and its composition calculated by recognized methods. The breast milk was expressed at 4-hour intervals throughout the experimental period and chemical analyses were made on the 24-hour composite samples. The detailed methods used in this study were described in preceding papers, where it was shown that these women chose diets that were liberal in quantity (21) and excellent with respect to quality and the proportions of the several nutrients (22). A comprehensive consideration of the volume output of breast milk (23) and its composition (24) has been reported elsewhere. The average daily milk output over a 60-week period during the fourth lactation for subject VI and the third for subjects VII and VIII was 3134, 2366, and 1419 cc., respectively. These women were apparently producing milk at their maximal capacity, as indicated by the ingestion of a liberal diet, the persistence in regular habits of good hygiene, and the regular, thorough, manual emptying of the breasts daily throughout the lactation period.

During this interval the body weight of subject VI decreased from 73.1 to 65.5 kilograms, that of subject VII decreased from 65.0 to 61.8 kilograms, and that of subject VIII increased from 70.4 to 81.8 kilograms.

Experimental

Calories. During intense lactation the caloric exchange by a woman who is maintaining her weight is a striking example of the transformation of ingested food into milk nutrients. Hoobler (25) has shown that mothers receiving 2670 and 2774 calories per day produced milk containing 1096 and 1296 calories, respectively, or 41 and 47 per cent of the total food calories. The notable ability of some women to convert food energy into milk energy is further shown in Table I and Chart 1. Subjects VI, VII, and VIII ingested daily dietaries with caloric values of 4300, 4600, and 3800, respectively, and secreted milk containing 2100, 1500, and 1120 calories, respectively.[1] The caloric content of the breast milk in these cases was equivalent to 50, 33, and 30 per cent of the total intake. The percentage distribution of calories between proteins, fats, and carbohydrates in the

[1] The mean food intakes cited in this paper represent the weighed food ingested on the days when breast milk was simultaneously collected.

diets of these women was in the ratio of 15:37:48, a proportion in agreement with that recommended by Rose (26, p. 25).

A comparison of the volume output with the caloric values of the three milks shows that the highest caloric value per liter, 770, was achieved by subject VIII, who produced the smallest volume of milk. However, the milk of subject VI had a caloric value of 680 per liter and that of subject VII only 660 per liter, although the former produced nearly 800 cc. more milk per day than the latter did. Hence, though the caloric value of the milk of these women showed individual variation, this variation was not necessarily related to volume.

TABLE I

ESTIMATED AVERAGE DAILY CALORIC BALANCE

Subjects	Total 24-hour			Per sq. m. body surface			Per kg. body weight*		
	VI	VII	VIII	VI	VII	VIII	VI	VII	VIII
Food, Cal.	4300	4600	3800	2500	2660	2090	65	73	49
Breast milk, Cal.	2100	1500	1120	1200	870	590	30	25	14
Excreta loss, Cal.	390	460	400	—	—	—	—	—	—
Surplus, Cal.	1810	2640	2280	1070	1530	1270	27.3	42.0	29.4
Milk value per liter, Cal.	680	660	770	Calories per sq. m. body surface per hour					
Average daily milk volume, cc.	3134	2366	1419	Food.			104	110	87
Outgo in milk as percentage of intake.	50	33	30	Surplus.			44.6	63.8	52.9

* Change in body weight during lactation in kilograms: VI, 73.1 to 65.5; VII, 65.0 to 61.8; VIII, 70.4 to 81.8.

Inasmuch as milk volume is the chief determinant of the total output of milk calories, the milk volume becomes a factor of moment in planning diets for lactating women. The active mother who is putting 2000 calories daily into milk must obviously receive a much more abundant diet than is needed by the mother (27, p. 602) who is secreting only 700 calories or less into breast milk. A continued inadequate caloric intake leads to a diminished milk flow. On the other hand, caloric overfeeding does not stimulate milk production (18, 28).

Breast milk has a variable caloric value, depending largely on its fat content and to a lesser degree on its concentration of carbohydrate and protein. Schlossman (13, p. 548) examined nineteen samples of milk from as many women and found an average caloric value per liter of 719 (maxi-

mum of 876 and a minimum of 567). Morse (29, p. 121) states that the caloric value of breast milk is usually cited as 782 per liter, although the individual milks vary greatly. The calories of breast milk are derived from the cleavage products of fats, carbohydrates, and proteins from either ingested food or maternal tissue. There appears to be a definite tendency,

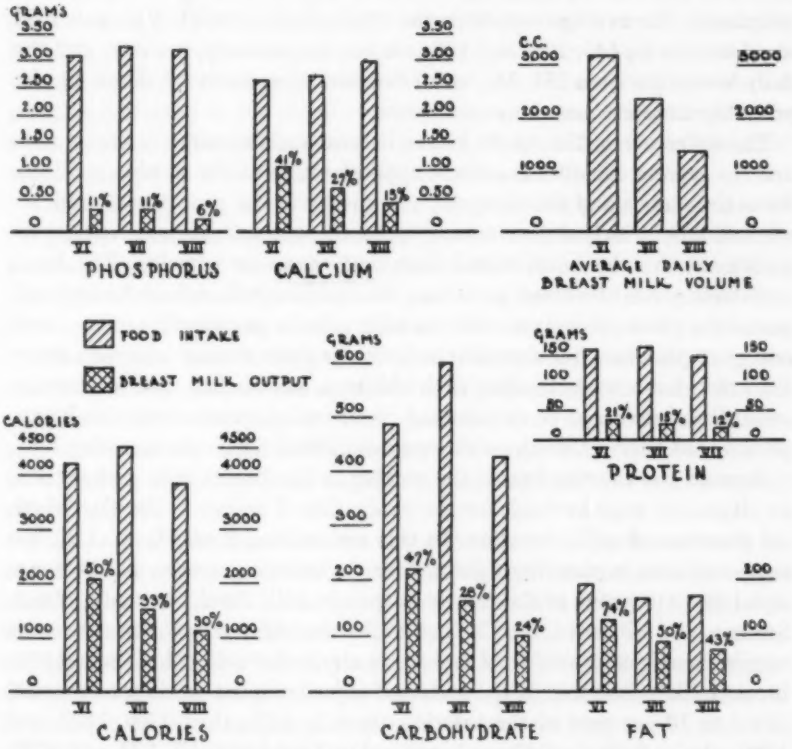

Chart 1.—Illustrates the average daily food intake and the outgo in breast milk of calories proteins, fat, carbohydrate, calcium and phosphorus.

especially in early lactation, to maintain milk secretion at the expense of the maternal caloric reserves (12, p. 496). Lusk asserts (13, p. 529) that lactation does not appear to increase the heat production.

> This is not strange [he writes] since the rearrangement of food materials in the preparation of milk depends on hydrolytic cleavages and syntheses which involve hardly any thermal reactions, and also because it is known that the secretory activity of a gland has no influence upon the total heat production of the body.

The calories contained in the excreta constitute an additional energy loss to the maternal body. The approximate amount of this decrement has been determined by the use of the following factors described by Lusk (13, p. 53) and by Murlin and associates (30): each gram of urinary nitrogen was considered to represent the loss of 7.9 calories and each gram of dry feces was taken as representing the loss of 6.2 calories. By this method of calculation the average output in the urine of subjects VI, VII, and VIII was found to be 140, 125, and 130 calories, respectively, per day, and the daily loss in the feces 254, 330, and 270 calories, respectively. These figures probably underestimate this caloric loss.

The difference in the caloric intake in food and the outgo in breast milk and the excreta constitutes a crude approximation of the surplus available for maintenance and the dissipation of energy in the processes of milk secretion. The principal determinate factors in the maintenance energy requirement are the basal metabolism and muscular activity. The basal metabolic needs of women have been studied in detail and can be approximated for these subjects as 1300 to 1500 calories per day. Since the exact energy requirement for muscular activity for these women who were doing their own housework, tending their children, and leading their customary social lives could not be determined, their maintenance needs can be approximated only within the wide range of 2200 to 3200 calories a day.

Beyond the calories lost to the mother in the breast milk and excreta, an allowance must be made for the dissipation of energy in the physiological processes of milk secretion. In this connection, Rose (31, p. 118) has suggested that in planning a dietary for the lactating woman an allowance equal to 10 per cent of the caloric output in milk should be made. Rand, Sweeny, and Vincent (32, p. 217) state that in addition to the maintenance requirement, the provision of two food calories for each calorie secreted in breast milk is desirable. If the maternal organism must sacrifice an amount equal to 10 per cent of the caloric output in milk, then 2300, 1600, and 1200 calories daily would have been used up by subjects VI, VII, and VIII, respectively, for that purpose, whereas the dissipation of a quantity equal to the caloric content of the milk would have required the use of 4200, 3000, and 2200 calories, respectively, beyond maintenance. Consequently, if these mothers were 90 per cent efficient in the transformation of food energy into milk energy, as assumed by Rose, 2000, 3000, and 2600 calories daily for subjects VI, VII, and VIII, respectively, would be available for maintenance; on the other hand, if they were only 50 per cent efficient, as assumed in the second dietary regimen, then 100, 1600, and 1600 calories daily, respectively, would have been available for maintenance. From the

above summary it would appear that the women of this investigation exhibited a high degree of efficiency in the transformation of food energy into milk. Subjects VI and VII, producing the largest quantities of milk (3134 and 2366 cc. respectively), lost 7.6 and 3.2 kilograms respectively in body weight during lactation, but this total loss in body weight extending over a 60-week period could not have been a conspicuous source of calories for physiological activities. The caloric overfeeding which would have prevailed if these women had taken the overly generous supply of two food calories for each breast milk calorie beyond maintenance requirements, a practice indicated in the dairy industry, has not been demonstrated to be advantageous either to the cow (33, p. 130; 34, p. 514) or to woman, and indeed may prove inimical to milk flow in woman (28, 35).

These findings on three women who are physiologically endowed with the ability to secrete from two to three times the amount of milk ordinarily produced by women, show that it would have been undesirable to follow the dictum of animal experiments in providing for their body needs. Differences in species, such as exist between the ruminant cow and woman, have to be considered in the physiological utilization of food calories in the secretion of milk and in the appraisal of lactation requirements. The results here recorded suggest an urgent need for more comprehensive observations on women and a closer scrutiny of the present empiric standards for lactation, which are based largely upon experiments on mammals other than man.

Fat. As would be expected, the concentration of fat in breast milk resembled the caloric concentration. The highest fat concentration was achieved by subject VIII, who produced the smallest volume of milk. The milk volume ratios for subjects VI, VII, and VIII were 15:12:7, and the corresponding milk fat values per liter were 40, 36, and 48 grams, respectively. It is a common observation in the dairy industry that the amount of milk fat tends to vary inversely with the volume output. Subject VII chose slightly less dietary fat and much more carbohydrate than the other two women, but she did not produce as rich a milk as they.

In the experiments reported here, milk fat represented an even greater percentage of the food fat (Table II) than was observed in the caloric exchange. Subjects VI, VII, and VIII secreted 128, 84, and 73 grams of fat into breast milk in a 24-hour period on food containing respectively 194, 167, and 170 grams of fat. Thus milk fat was equivalent to 74, 50, and 43 per cent of the total intake in these respective cases. The quantity of fat remaining for utilization in metabolism amounted to 0.9, 1.2, and 1.3 grams per kilogram of body weight, which can be regarded as an adequate

but not liberal allowance. In connection with fat consumption, Rose (26, p. 25) states that "The average man's maximum capacity is said to be about 200 grams per day and he does not seem to maintain the best health with less than about 75 grams per day."

TABLE II

AVERAGE DAILY FOOD INTAKE AND BREAST MILK OUTPUT

Subjects	Proteins			Fats			Carbohydrates		
	VI	VII	VIII	VI	VII	VIII	VI	VII	VIII
Food, grams	160	165	150	194	167	170	488	600	425
Breast milk, grams	33	25	18	128	84	73	222	163	99
Surplus, grams	128	141	133	60	76	100	268	437	324
Surplus, per kilogram body weight	2.1	2.2	1.7	0.9	1.2	1.3	4.0	6.8	4.1
Breast milk in percentage of food intake	21	15	12	74	50	43	47	28	24
Breast milk grams per liter	10	10	12	40	36	48	70	70	68

Meigs (18) states that there is little doubt that amino acids, phosphatids, and glucose "are really the general currency of metabolism—the chemical forms in which the proteins, fats, and carbohydrates are distributed by the blood to all of the organs and tissues of the body." Meigs reviews both the experiments indicating that the phosphatids are the direct precursors of milk fat and the conflicting data of Foa (36), who contends that milk fat is formed from the triglycerides of blood. Regardless of the precise precursor of milk fat, it appears that under ordinary circumstances it is formed from food lipids, and hence is influenced in composition by the character of the diet (37). If the dietary fat is inadequate, food carbohydrates, proteins, and deposit or tissue fat will be used as a source of milk fat. The experiments of Maynard and McCay (37) demonstrated that the fat secreted by the milking cow and goat on a low-fat ration had a lower iodine number than did that produced on a normal ration.

Fat is the most variable constituent of milk. Several factors, including the level of dietary fat or protein and the milk volume, may affect it quantitatively and qualitatively. The literature reviewing the influence of dietary fat on the quantitative secretion of milk fat shows seemingly contradictory and inconclusive results.

Carbohydrate. In accordance with similar observations on dairy animals, little difference was found in the concentration of lactose in the milks of the women of the present study, although there were individual variations in milk yield and in carbohydrate consumption. The lactose values per

liter of breast milk were approximately 70 grams for all three women. Subjects VI, VII, and VIII chose average daily diets containing respectively 488, 600, and 425 grams of carbohydrate, and were able to secrete 222, 163, and 99 grams of lactose into their milk. These quantities of lactose were equivalent to 47, 28, and 24 per cent of their respective carbohydrate intakes. The surplus of food carbohydrates over lactose secreted amounted to 4.0, 6.8, and 4.1 grams per kilogram of body weight for subjects VI, VII, and VIII, respectively.

There is a great deal of evidence to show that lactose is derived from blood sugar. Observations on lactating animals have shown that lactose is the most constant of the main organic constituents of milk and that unlike proteins and fats its concentration is not easily affected by changes in the ration.

Protein. According to Sherman (38), an allowance of 1.0 gram of protein per kilogram of body weight provides liberally for adult maintenance. The surplus food protein beyond that given out in breast milk by subjects VI, VII, and VIII amounted respectively to 2.1, 2.2, and 1.7 grams per kilogram of body weight. There was thus a considerable amount of protein for dissipation in milk secretion. Rose (39, p. 452) and McLester (40, p. 273) state that the human mother is only about 60 per cent efficient in the secretion of milk protein and that, accordingly, in lactation two grams of dietary protein, in addition to the maintenance intake, should be ingested for each gram of protein in breast milk, or 0.75 grams of additional protein for each ounce of milk produced. After deducting the amount of protein used in milk secretion, according to the above proposals, subjects VI, VII, and VIII would have had respectively 94, 115, and 114 grams of protein left for the remaining body functions.

Subjects VI, VII, and VIII chose liberal diets which contained an average of 160, 165, and 150 grams of protein per day, respectively (Table II). They secreted 3134, 2366, and 1419 cc. of milk daily, containing 33, 25, and 18 grams of protein, representing 21, 15, and 12 per cent respectively of the protein intake. The excellent quality of the ingested protein is shown by the fact that 55 to 75 per cent of it was derived from animal sources, chiefly from milk, while cereals constituted the largest fraction of the vegetable portion.

The concentration of protein in breast milk was similar for all subjects (10 to 12 grams per liter), but again the milk of the smallest producer, subject VIII, showed a 20 per cent higher value than that of the other women.

It is difficult to determine the protein requirement of an individual, since it depends upon the amino acid content of the assimilated protein,

the number of non-protein calories, and the composite specific amino acid needs of all body tissues under the particular conditions existing at the time. It is quite probable that the amino acid requirements for special body functions such as growth, maintenance, or lactation are different in quantity and quality, so that the efficiency of protein utilization in each case will depend on the specific demands of the tissues and the particular proteins involved. In addition, the effectiveness of the preparation for lactation during pregnancy constitutes a decisive factor in the success of milk secretion. Because of the multiplicity of determinate influences, it becomes difficult to set a physiological minimum for any body function and, indeed, it is impossible to state a quantitative protein requirement for complicated physiological functions such as those involved in lactation superimposed on maintenance. On the other hand, the provision of an adequate protein intake during lactation is of importance, since it has been shown that milk protein is ultimately derived from food protein and that the level of dietary protein affects the quantity of milk secreted and, within limits, its composition (18, 19).

The milk proteins are derived from the free amino acids of the blood. It has been shown that the quantity and quality of the amino acids in the circulating blood are changed by diet, by the varying nitrogen demand of the tissues, and by the supply of non-protein calories. These changes have a marked influence on milk secretion and, in addition, protein metabolism is intimately related to the metabolism of carbohydrate and fat. Meigs (18) states that

> A change in the carbohydrate of the diet may affect the amino-acids of the blood, and thereby the secretion of milk protein; and a change in the protein of the diet may affect the secretion of milk fat—probably again through a change in the amino-acids of the blood.

The women of this study were successful milk producers. Their daily dietaries during pregnancy were liberal in calories and included 90 to 133 grams of protein of high biological value. All but five of the twenty-five nitrogen metabolic balances during pregnancy and lactation were positive. There is evidence to indicate that nitrogen retention beyond that required by the fetus and adnexa favors success in lactation (41 to 44). During the period of increased maternal demands, these subjects ingested larger quantities of food, including protein of excellent quality.

Calcium and Phosphorus. In accordance with findings from experimental work on animals, the calcium and phosphorus values of the breast milk for the three women of these studies were found to be fairly constant. The intake and outgo of these substances are summarized in Table III, where it is shown that the calcium in breast milk amounted to 1.17, 0.75, and

0.45 grams daily for subjects VI, VII, and VIII, respectively, and was equivalent to 41, 27, and 15 per cent of the total calcium intake of 2.71, 2.82, and 3.08 grams daily. The calcium intake stood in inverse relation to milk volume and hence to the total excretion in breast milk.

TABLE III
AVERAGE DAILY MINERAL INTAKE IN FOOD AND OUTGO IN BREAST MILK

Subjects	Calcium			Phosphorus		
	VI	VII	VIII	VI	VII	VIII
Food, grams	2.71	2.82	3.08	3.15	3.31	3.28
Breast milk, grams	1.17	0.75	0.45	0.36	0.35	0.20
Surplus, grams	1.54	2.08	2.63	2.78	2.96	3.08
Surplus per kilogram body weight	0.023	0.033	0.033	0.041	0.046	0.038
Milk content as percentage of intake	41	27	15	11	11	6
Milk value, grams per liter	0.36	0.32	0.32	0.12	0.16	0.14

Similarly the phosphorus outgo in milk of 0.36, 0.35, and 0.20 grams per day was equivalent to 11, 11, and 6 per cent of the phosphorus intake of 3.15, 3.31, and 3.28 grams per day for subjects VI, VII, and VIII, respectively. The phosphorus intakes were likewise in inverse relation to the milk yield and to phosphorus outgo in breast milk.

Sherman (38) found the adult minimum maintenance requirements to be 0.0064 grams of calcium and 0.0126 grams of phosphorus per kilogram of body weight. In order to insure a factor of safety he suggested that 50 per cent more of each element, or 0.0096 grams of calcium and 0.0189 grams of phosphorus per kilogram of body weight, be regarded as an adequate allowance for these substances. The surplus of calcium intake over calcium outgo in milk was 0.023, 0.033, and 0.033 grams per kilogram of body weight. The phosphorus intake exceeded the phosphorus outgo in the milk by 0.041, 0.046, and 0.038 grams per kilogram of body weight for subjects VI, VII, and VIII respectively.

Although their diets contained liberal quantities of calcium and phosphorus, chiefly from foods such as milk, eggs, and cheese, from which these elements are usually well assimilated, the women of this investigation were unable adequately to absorb or utilize calcium and phosphorus while maintaining a high level of milk flow. Only one of thirteen metabolic calcium balances during lactation was positive, while four of thirteen metabolic phosphorus balances during this period were negative (16, 17). The negative calcium balances were largely due to the excessive excretion of that element in the feces. In three of thirteen balances the fecal excretion of cal-

cium exceeded the intake, and in eight of thirteen the fecal excretion was more than 75 per cent of the intake.

The consideration of the dietary calcium and phosphorus intake and the outgo of these substances in milk is particularly pertinent with reference to the subjects of this study, who were secreting large quantities of milk and were found to have negative calcium and phosphorus balances while maintaining this high level of milk flow (17). Meigs and Cary (12, p. 511) state that studies of calcium and phosphorus metabolism have shown that

> the bones serve as a great storehouse for these elements, which can store up a surplus in periods of plenty and yield it up again in periods of need. And again,
>
> If either calcium or phosphorus is deficient in the food of a milking animal, she will take both elements from her bones and use whichever one is necessary to keep up the normal composition of the milk.

From these data on the nutritive performance of three women during lactation it is evident that providing an adequate diet is not the only essential; more satisfactory ways and means of inducing better digestion, absorption, and assimilation of the food materials taken into the body must be devised if lactation is to leave the maternal body unimpaired.

Summary

A quantitative study of the self-chosen diets of three multiparae is reported. The nutritive requirements during 60 weeks of intensive milk flow are considered, with particular reference to the relationship between the intake of food calories, proteins, fat, carbohydrates, calcium, and phosphorus and the outgo of these components in breast milk.

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THE IRON REQUIREMENT OF THE PRE-SCHOOL CHILD*

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AT THE time this study was undertaken, no information on the iron requirement of young children based on balance experiments was available. Since then there has appeared the report of an iron-balance study by Rose, Vahlteich, Robb, and Bloomfield (4) on a girl 31 months of age.

The present investigation was carried out as a typical balance experiment in which the utilization of iron at two levels of intake was compared. Four healthy pre-school children, one boy and three girls, whose ages ranged from 35 to 56 months, served as subjects. The experiment was divided into two periods of 5 days each. In period I a diet low in iron was given and in period II a diet high in iron was supplied. All food consumed by these children over the 10-day period of the experiment was weighed and prepared by one of us. Splendid coöperation was obtained from the parents who understood and appreciated the precautions necessary in order to make the investigation a success.

On the basis of the figures in Sherman's tables (5), the diet in period I was calculated to contain 5 milligrams of iron and the diet in period II, 8.5 milligrams. The results of the analyses of the various foods included in the diet showed, however, that the iron intake actually fell considerably below this estimated amount, the analyses showing 3.25 milligrams and 6.5 milligrams respectively for the 2 diets, or 35 per cent and 24 per cent less than the calculated amounts. This observation is in accord with that of Rose, Vahlteich, Robb and Bloomfield who report that the iron content of their diet, as calculated from Sherman's tables, was over 30 per cent higher than the actual amount as determined by analyses. In the present study all foods which contributed significant amounts of iron to the diet were analyzed. The results of these analyses appear in Table I.

The diet during the period of low iron intake consisted of orange juice, puffed rice, egg, potato (peeled), white bread, canned peaches, canned tomatoes, canned string beans, rice, milk, cream, butter, and sugar. Dur-

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ing the period of high iron intake the diet included: raisins, shredded wheat biscuit, white and graham bread, chopped beef, potato (peeled), carrots, prunes, canned peas, lettuce, egg, milk, cream, butter, sugar, and cornstarch. The variety and combination of foods was such that no difficulty was experienced in getting the children to eat the same foods for the 5-day period. Distilled water was allowed in unlimited quantities for drinking purposes and was also used in the food preparation. The diet, as planned, was based on the accepted dietary standards for children.

TABLE I
IRON CONTENT OF FOOD MATERIALS PER 100 GRAMS EDIBLE MATERIAL

	Analysis 1 mgm.	Analysis 2* mgm.
Bread, graham	1.19	—
Bread, white	0.67	—
Carrots	0.39	0.63
Egg, cooked	1.92	—
Lettuce	0.31	0.56
Meat, beef, round	3.45	3.02
Milk, whole	0.18	—
Orange juice	0.14	0.24
Peaches, canned	0.25	—
Peas, canned	1.23	—
Potatoes, peeled	0.39	0.52
Prunes	2.33	1.72
Raisins	4.04	2.43
Shredded wheat biscuit	3.05	—
String beans, canned	0.38	—
Tomatoes, canned	0.16	—

* Where no figure appears in this column, the same analysis covers both experimental periods.

The first two days on each of the experimental diets were used as a preliminary period to allow for an adjustment of the iron balance to this level of intake. During the following 3 days, collections of urine and feces were made. The urine samples were measured and stored in sealed glass bottles, using toluol as a preservative. For the marking of the feces, a 0.2 gram carmine capsule was given at the beginning and end of the collection period. The feces were covered with a mixture of concentrated hydrochloric acid and 95 per cent alcohol (1:3) and dried on the steam bath in a covered evaporating dish. When dry the feces were weighed, ground in a mortar, thoroughly mixed, and stored in sealed glass bottles.

All foods which have good keeping qualities were purchased in one lot for the entire experiment. Every effort was made to have the foods which

were purchased from day to day as nearly uniform in composition as possible. Samples for analyses were always taken at the time the foods were weighed for the diet. The composite food samples thus obtained were dried in covered evaporating dishes. The air-dried food was weighed, ground in a mortar, and, after thorough mixing, was stored in sealed glass bottles.

Analyses of food, feces, and urine for iron were made in triplicate using the potassium permanganate titration method as modified by Murray (3). As a preliminary preparation of the urine, a modification of the Wolter method (Hawk and Bergeim, (1)) was used. Three cubic centimeters of concentrated nitric acid were added to a 150 cc. sample of urine. This was allowed to evaporate to a low volume on the steam bath. The residue was then transferred to a crucible and charred over a very low flame. Ashing was completed in the muffle furnace at 500°C. The size of the samples of food and feces used for analyses was such as to insure approximately 0.2 milligram of iron in each sample. Correction was made for the iron content of all reagents used. Every precaution was taken to avoid iron contamination of materials by keeping them constantly covered during storage, preparation, and analytical procedures and by using only such utensils as contain no iron.

Discussion

The average daily excretion of iron in the urine and feces of the four subjects is shown in Table II. It will be noted that there was a considerable difference in the amount of urinary iron excreted by the 4 subjects and also that there was a distinct increase in the amount of iron in the urine during the period of high iron intake in three of the subjects. The average amount of urinary iron reported by Rose, Vahlteich, Robb, and Bloomfield for the subject in their experiment was 0.20 milligram on an iron intake of 4.64 milligrams; a figure which is intermediate when compared with the four subjects of this experiment on the low-iron diet, but low when compared with the figures in this study on the high-iron diet. The daily fecal output of iron during the period of low-iron intake was fairly uniform for the four subjects in this study, the range being from 1.34 to 2.00 milligrams per day. Greater variation was observed during the period of high-iron intake, the range being 2.07 to 3.21 milligrams. The total daily output on iron averaged 2.07 milligrams during the period of low iron intake, and 3.29 milligrams during the period of high-iron intake. All of the children were in positive balance throughout. The average daily iron retention during the period of high-iron intake was 2.02 milligrams greater

than during the period of low-iron intake, or an increase of 170 per cent over the amount retained during the period of low-iron intake.

Obviously, the iron intake in period I did not meet the needs of these children for optimal storage of this element, since a much greater amount was retained during the period of high-iron intake. If the level of iron excretion in period I may be taken as an index of the maintenance requirement of these children, it would appear that approximately 2.1 milligrams

TABLE II

AVERAGE DAILY INTAKE AND OUTPUT OF IRON

PERIOD I—LOW IRON INTAKE

Subject	Sex	Age	Energy intake	Iron intake	Iron output in urine	Iron output in feces	Total iron output	Iron retention
		mo.	cal.	mgm.	mgm.	mgm.	mgm.	mgm.
A	Male	55	1280	3.12	0.11	1.74	1.85	1.27
B	Female	35	1006	2.74	0.13	1.34	1.47	1.27
C	Female	46	1349	3.52	0.48	2.00	2.48	1.04
D	Female	56	1429	3.64	0.58	1.90	2.48	1.16
Average		48	1266	3.25	0.30	1.75	2.07	1.19

PERIOD II—HIGH IRON INTAKE

A	Male	55	1352	6.61	0.22	2.07	2.29	4.32
B	Female	35	1296	6.47	0.30	2.93	3.23	3.24
C	Female	46	1426	6.42	0.81	3.09	3.90	2.53
D	Female	56	1444	6.50	0.53	3.21	3.74	2.76
Average		48	1380	6.50	0.49	2.80	3.29	3.21

of iron are needed daily for this purpose or, on the basis of body weight, the requirement would be 0.12 milligram per kilogram. Calculating the maintenance needs of children of this age on the basis of the adult requirement of 10 milligrams iron per 70 kilograms body weight (Sherman, 5) would give a maintenance requirement of 2.4 milligrams per day. This figure agrees so closely with the maintenance requirement of 2.1 miligrams observed in this study as to suggest that on the basis of body weight, the maintenance need of the child is similar to that of the adult. The maintenance requirement observed by Rose, Vahlteich, Robb, and Bloomfield on their subject was 5.70 milligrams per day or 0.41 milligram per kilogram, a requirement which is very much higher than that of any of the subjects in this study (Table III).

Inasmuch as the excretion of iron during period II was considerably above the level of excretion in period I, it would seem that this higher level of intake more than supplied the needs for optimal storage. It is recognized, of course, that a part of this increase may result from a failure of absorption of iron due to its unavailability and to that extent it does not represent a surplus. Assuming, however, that optimal storage occurred during this period of high-iron intake, it would appear from the results of

TABLE III

IRON REQUIREMENT FOR MAINTENANCE AND GROWTH BASED ON DATA IN THIS STUDY

Subject	Energy intake	Weight	Iron Requirement				
			For maintenance	For growth	Total requirement for maintenance and growth	Total requirement per kg. of body weight	Total requirement per 100 Cal. of food intake
	cal.	kgn.	mgm.	mgm.	mgm.	mgm.	mgm.
A	1316	16.40	1.85	4.32	6.17	0.377	0.475
B	1151	15.19	1.47	3.24	4.71	0.310	0.270
C	1387	18.60	2.48	2.52	5.00	0.274	0.361
D	1436	18.49	2.48	2.76	5.24	0.283	0.365
Average	1322	17.15	2.07	3.21	5.28	0.308	0.392

this study that the iron requirement for growth was approximately 0.2 milligram per kilogram. The iron requirement for maintenance and for growth of the four children based on the above assumptions is shown in Table III. The calorie intake is the average for each child over the two experimental periods.

On the basis of the findings in this study, the following iron allowances for pre-school children may be tentatively suggested: for maintenance, 0.12 milligram per kilogram; for growth, 0.20 milligram per kilogram; or a total of 0.32 milligram per kilogram. Since the results of the food analyses in this experiment indicate that the amount of iron in different foods may be considerably less than the most commonly used figures, and also since there are pronounced individual differences in the iron requirement of children, it would seem that a margin of safety of at least 50 per cent above this calculated requirement of 0.32 milligram per kilogram should be allowed in planning the dietaries of pre-school children. This would make a standard allowance of 0.48 milligram per kilogram. On this basis

the total daily iron requirement of the children in this study would be 8.23 milligrams or 0.62 milligram per 100 calories. These figures are in close agreement with the allowance of 8.5 milligrams or 0.76 milligram per 100 calories suggested by Rose, Vahlteich, Robb, and Bloomfield, and also with the iron intake of children in private homes observed by McKay (2). She reports an average of 8.17 milligrams of iron in the diets of preschool children in private homes as compared with 4.37 milligrams in the diets of children in an institution.

Summary

1. Two iron-balance studies were conducted using four healthy children, one boy and three girls, ranging in age from 35 to 56 months. Iron retentions at two levels of intake were compared.

2. The results of the food analyses indicate that the iron content of foods may show considerable variation from the most commonly used figures. Diets which were planned to contain 5 and 8.5 milligrams of iron actually contained only 3.25 and 6.5 milligrams respectively.

3. On a diet containing 3.25 milligrams of iron, an average of 1.2 milligrams was retained daily, whereas, on a diet containing 6.5 milligrams of iron, 3.2 milligrams, or nearly three times as much as during the period of lower iron intake, were retained.

4. The observed maintenance need of the children in this study was approximately 0.12 milligram per kilogram. It would appear from these results that, on the basis of body weight, the maintenance requirement of the child is similar to that of the adult.

5. The iron requirement for growth, as observed in this study, was approximately 0.2 milligram per kilogram.

6. In view of the differences observed between the actual iron content of the food used and the amounts as calculated from Sherman's tables, and also the pronounced individual differences in the iron requirement of children, it is suggested that a 50 per cent margin of safety be allowed above this observed total requirement of 0.32 milligram per kilogram. This would make a standard allowance for children of this age of 0.48 milligram per kilogram, or 0.62 milligram per 100 calories, or a total of 8.23 milligrams per day.

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DIETARY REQUIREMENTS FOR FERTILITY AND LACTATION

XXIV. FURTHER STUDIES ON THE SPECIFIC EFFECT OF VITAMIN B ON LACTATION*

By

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IN a recent communication it was demonstrated that when albino rats are transferred on the day of birth of their litters from our stock diet to a ration deficient only in vitamin B, vitamin G having been furnished in abundance by the introduction of 10 per cent autoclaved Northwestern yeast in the ration, infant mortality to the extent of 70 to 100 per cent was encountered in one to two weeks. When, however, litter mate lactating animals were restricted to the same daily food and water intake of the same diet but which contained 10 per cent untreated Northwestern yeast, thus providing adequate amounts of both vitamins B and G, there was 80 to 100 per cent survival of young, and growth of litters actually took place but at a subnormal rate. It, therefore, became apparent that vitamin B, in addition to stimulating the appetite, exerts a specific beneficial influence on lactation unrelated to food intake.

In this study additional experimental evidence was secured which corroborates the previous findings of Sure and Walker (1). A few experiments were conducted in pairs, the majority, however, having been carried out in triads. The object was to produce vitamin B deficiency in the lactating rats and nursing young gradually during the middle or later part of lactation, aiming particularly at prolonged maintenance, so that such baby rats could be used for blood studies in lipid metabolism without the possible disturbance of the complicating factor of catabolism of body tissue. The modified technic was carried out as follows:

Lactating rats were transferred on the day of the birth of their litters, reduced to 6 in number, from stock diet No. 1 (2) to Ration 1865, satisfactory in every respect with the exception of vitamin B, of the following composition: Casein,[1] 20; salt mixture, 185 (3), 4; autoclaved yeast,[2] 10;

* Research paper No. 203, Journal series, University of Arkansas.

[1] Purified by extraction for 10 days with acidulated water.

[2] Northwestern dehydrated yeast was autoclaved in shallow glass Pyrex dishes for 6 hours at 20 pounds pressure.

butter fat, 10; dextrin, 56. Litter mates were restricted to the same amount of water and food of the same diet, but the yeast contained in the ration was untreated. The only limiting factor on such a dietary regime, since the plane of nutrition was controlled, was vitamin B destroyed during the process of autoclaving. Another group of litter mates was fed the same ration containing 10 per cent of the untreated yeast but the daily food and water intake was unrestricted. The difference between the second and third groups must then be due to inanition, since the only limiting factor was the plane of nutrition. In order to prevent early infant mortality and produce prolonged maintenance of the young, the mother on the vitamin B-deficient ration was given from the day of delivery of her litter a supplementary allowance of 500 mg. of Northwestern yeast separately from the ration. Such an administration of yeast was also given to the lactating rats of the second and third groups, so as to keep all the dietary factors constant. After the pathological litters had reached a collective weight of 110 to 120 gms., the yeast was either gradually or entirely removed from the mothers as well as from the control animals. The subsequent slow growth in the pathological nursing young can be explained by the securing of stored vitamin B from the mothers. In a few cases when it was anticipated, after the young began eating, that traces of vitamin B may have been furnished by the yeast in the ration, which was autoclaved for 6 hours at 20 pounds pressure, the yeast was replaced by another batch autoclaved for 8 hours at 25 pounds pressure according to a recent suggestion by Salmon (4).[3]

From our numerous records we have selected four typical cases, one pair and three triads, which are shown graphically in Charts 1 to 4 inclusive.

An examination of curves 1 and 2 clearly shows that up to the 9th day no difference is evident in the lactation efficiency index produced by vitamin B in the diet. However, from then on the effect of vitamin B *per se* becomes more and more pronounced, so that on the 36th day the pathological litter on the maternal diet, deficient in vitamin B, weighed 120 gm. and the control litter, on the same maternal diet, but containing an abundance of vitamin B, and restricted to the same daily food and water intake, weighed 180 gm. On the 46th day the pathological litter was reduced to 3 which were in a dying condition, while in the control litter there were 5 young left on that day in an apparently healthy, vigorous condition.

[3] We have evidence that the yeast under this more drastic treatment at its natural pH supplied an abundance of the vitamin G growth-promoting factor.

The specific influence of vitamin B on lactation is very marked, as designated by curves 1 and 2. For a period of 23 days while the pathological litter had practically maintained its weight, the control litter, whose maternal diet on the same plane of nutrition contained vitamin B, gained 70 gm. The specific effect of vitamin B on lactation at the termination

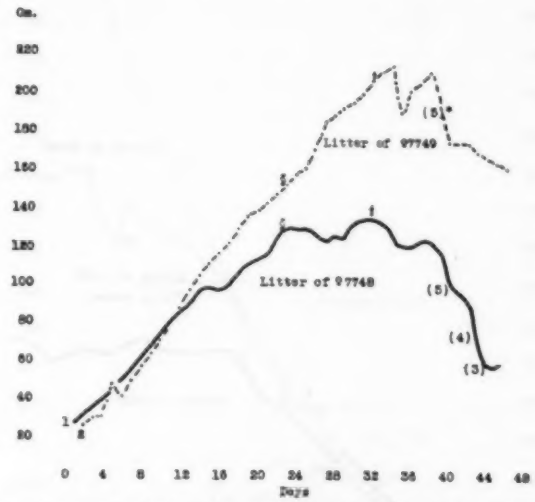

CHART 1. Curve 1 represents the lactation record of ♀ 7748, the diet of which was satisfactory in every respect but deficient in vitamin B, 10 per cent autoclaved Northwestern dried yeast serving as the source of vitamin G; Curve 2, the lactation record of ♀ 7749, on the same diet containing 10 per cent untreated Northwestern yeast, and, therefore, supplying an abundance of both vitamins B and G. Since ♀ 7749 was restricted to the same daily food and water intake as that consumed by its litter mate, ♀ 7748, the limiting factor in the diet of lactating rat 7748 was vitamin B. Since it was desired to furnish to lactating ♀ 7748 a diet inadequate rather than entirely deficient in vitamin B, she was given from the day of the birth of her litter a daily supplement, separately from the ration, of 500 mg. Northwestern yeast which was also furnished at the same time to ♀ 7749. At point "§" the yeast was reduced to 300 mg. daily and at point "†" the administration of yeast was discontinued. Figures in parentheses represent the number of young left on that particular day. Keeping the plane of nutrition constant, the specific effect of vitamin B on lactation becomes apparent as is demonstrated in this and the succeeding charts.

* One young was accidentally killed in closing door of cage, leaving 5 in the litter.

of the experiment is expressed by the difference in the collective weights of litters of ♀ 7788 and of ♀ 7787, which is 240 gm. − 153 gm. = 87 gm. The inanition effect is expressed by the difference in lactation performance of ♀ 7788, which was restricted, and ♀ 7789, which was unrestricted on the same complete diet. Although ♀ 7788 was actually able to wean her litter of 5 young, she was able to do so only 8 days later than

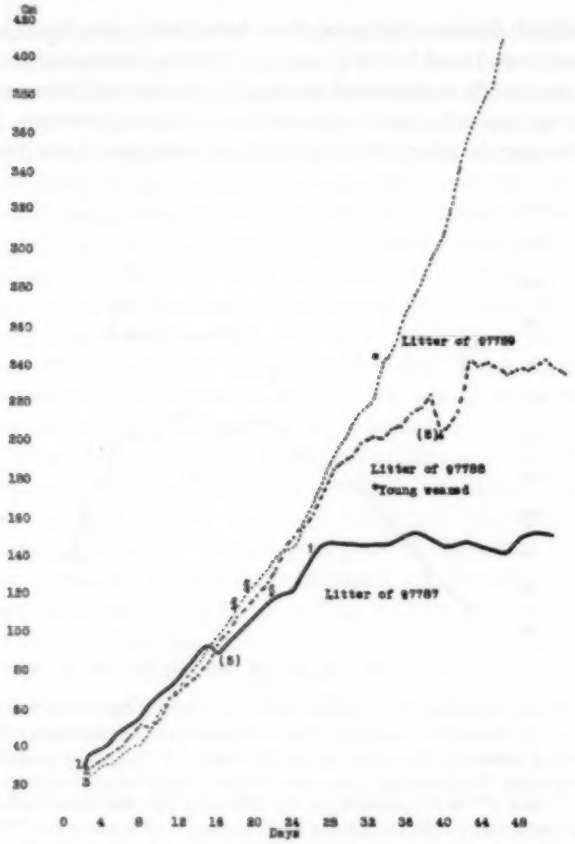

CHART 2. Curve 1 represents the lactation record of ♀ 7787, the diet of which was deficient only in vitamin B, 10 per cent Northwestern yeast autoclaved for 6 hours at 20 pounds pressure serving as the source of vitamin G; Curve 2, the lactation record of litter mate, ♀ 7788, on the same diet containing 10 per cent of the yeast untreated, and, therefore, furnishing an abundance of vitamins B and G; Curve 3, the lactation record of ♀ 7789 on the same daily food and water intake as its litter mate, ♀ 7788, but was unrestricted to its daily food and water intake. All the lactating rats received from the day of the birth of their litters 500 mg. daily of the Northwestern yeast separately from the ration. At point "§" the daily administration of the 500 mg. yeast was discontinued. At point "†" the autoclaved yeast in the ration of ♀ 7787, which was heated for 6 hours at 20 pounds pressure, was replaced by a batch autoclaved for 8 hours at 25 pounds pressure. The latter insured the absence of any traces of undestroyed vitamin B but still provided an adequacy of vitamin G for the requirements of nursing young.

Figures in parentheses represent the number of young left on that particular day.

The difference in lactation performance between females 7787 and 7788 is due to the specific effect of vitamin B. The difference in lactation efficiency between females 7788 and 7789, however, is due to the difference in the plane of nutrition.

her litter mate, ♀ 7789, on the same unrestricted diet. The difference in the collective weight of the litters at weaning time was 39 gm. The difference after weaning is much more accentuated as shown by curves 2 and 3.

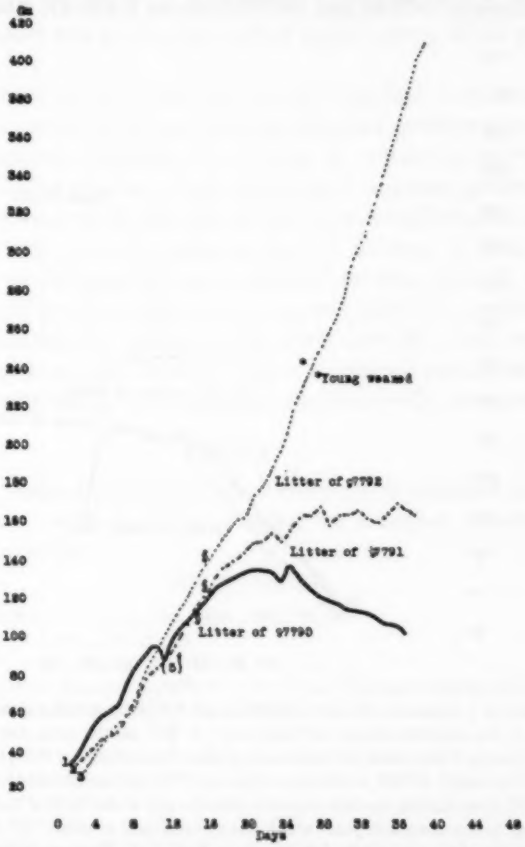

CHART 3. Curve 1 represents the lactation record of ♀ 7790 on a diet deficient only in vitamin B; Curve 2, the lactation record of a litter mate, ♀ 7791, on the same diet supplemented with an abundance of vitamin B, but restricted to the same plane of nutrition as ♀ 7790; Curve 3, the lactation record of litter mate, ♀ 7792, on the same diet as ♀ 7791 but unrestricted as to its daily food and water intake. From the day of the birth of their litters all these lactating rats received a daily supplement of 500 mg. of Northwestern yeast. At point "§" the yeast was removed. Sign "†" indicates that one young died leaving 5 in the litter. At point "•" the litter of ♀ 7792 was weaned. The specific effect of vitamin B and the influence of under nutrition are quite marked.

The specific effect of vitamin B on lactation does not become apparent in this group until the 13th day. From then on, ♀ 7790, on the vitamin B

deficient ration, lost one young and subsequently the litter of the female continuously lost weight until the 36th day when the experiment was terminated. The specific effect is expressed by the difference in the collective weight of litters of ♀ 7790 and ♀ 7791 (curves 1 and 2), which is 60 gm.

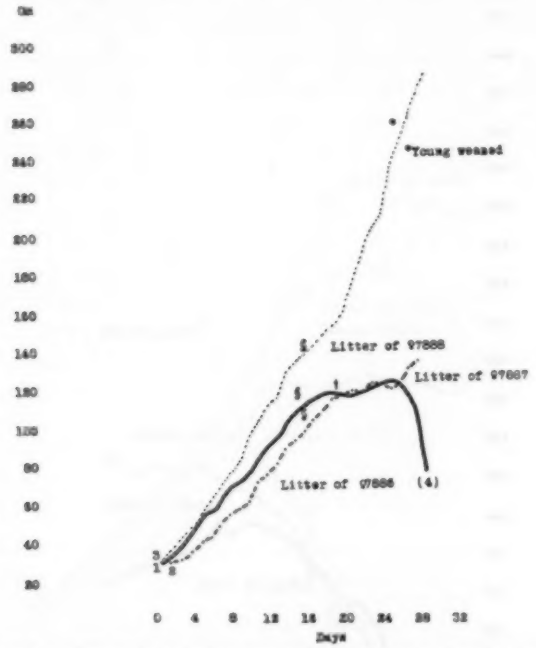

CHART 4. Curve 1 represents the lactation record of ♀ 7886 on a ration deficient only in vitamin B; Curve 2, the lactation record of litter mate, ♀ 7887, on the same diet fortified with an abundance of vitamin B but restricted to the same plane of nutrition as ♀ 7886; Curve 3, the lactation record of litter mate, ♀ 7888, on the same diet as ♀ 7887 but unrestricted to its daily food and water intake. All these nursing mothers received from the day of the birth of their litters a supplement of 500 mg. of Northwestern yeast which was discontinued at point "§." Number in parentheses indicates that four young were left out of six. Two of the four remaining young were in a dying condition. At point "*" the litter of ♀ 7888 was weaned. The specific effect of vitamin B on lactation and the influence of inanition on rearing of young are also quite pronounced.

The inanition effect at weaning is demonstrated by the difference in the collective weight of litters of ♀ 7791 and ♀ 7792 (curves 2 and 3), which is 80 gm. On the day the experiment was discontinued, the inanition effect was 240 gm.

In this particular group the specific influence of vitamin B on weaning of young did not become evident until the 29th day of lactation when

♀ 7886, on the vitamin B deficient ration, lost 2 young with 4 remaining young in a dying condition. On the same day the litter mate, ♀ 7887, was nursing all her 6 young which were in an apparently vigorous condition. The day the litter of ♀ 7888, on the unrestricted control diet, was weaned, there was an inanition effect to the extent of 120 gm. (curves 2 and 3).

In this connection, we would like to point out that very frequently we encounter what would seem as irregular lactation performance in case of litter mate controls receiving the vitamin B containing restricted diet. Occasionally for 7 to 10 days their litters show a poorer growth than the animals on the vitamin B deficient ration. This is explained as follows: In starting three litter mate females on days of delivery of their young on such studies, we frequently were compelled to take animals varying as much as 60 to 80 gm. in weight, and have found that the smaller animals, which were occasionally placed on the restricted diet, had considerably less vitamin reserves; and for that reason a delayed manifestation of the specific effect of vitamin B on lactation was frequently encountered.

Summary

Further evidence is submitted that vitamin B, in addition to stimulating the appetite, exerts a specific influence on lactation, unrelated to the food and water intake.

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THE SPECIFIC EFFECT OF VITAMIN B ON GROWTH*

By

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IN 1926 Drummond and Marrian (1) presented experimental evidence from which they drew conclusions that the physiological effects of vitamin B deficiency are essentially those of inanition or starvation. The lessened food intake, loss in weight, and fall in body temperature, they asserted, are all entirely the manifestations of inanition. The inference drawn, therefore, was that vitamin B produces growth only indirectly through stimulating the appetite. A careful analysis of the data of these investigators hardly justifies their sweeping conclusions as to the rôle of vitamin B in growth. In the first place they stated:

> A series of rats were fed daily on a complete artificial diet, plus 5 per cent yeast extract, in amounts of the order that it was estimated would have been voluntarily consumed had the animals been given an ample supply of the vitamin B-deficient ration. Control groups provided with an unlimited supply of the complete diet and the deficient ration were also observed. The general result of these tests was that the groups fed on the restricted ration of the complete dietary (approximately 1 gm. daily per 10 gm. body weight) maintained both body weight and temperature, whilst occasional estimations of oxygen consumption yielded values within the normal range.

In the text of the article the statement is definitely made that, on the same daily food intake, animals receiving in addition vitamin B in the form of a yeast extract, did not show the symptomatology of inanition fever or loss of body weight; yet, in the summary of the paper these English investigators concluded that . . . "in the case of rats, at any rate, the nutritive failure following a deficiency of vitamin B is virtually identical with that of starvation." The additional experiments, from which Drummond and Marrian based their conclusions, were carried out as follows: To quote:

> A number of parallel experiments to those just described were carried out in which animals of similar weight were given daily from 0.5–1 gm. of yeast extract as their sole food together with ample supplies of water. The amount of utilizable food, from the standpoint of calories, supplied in this yeast extract was negligible. In no essential did the behaviour of the animals in this group differ from those maintained on water alone. The provision of ample supplies of vitamin B neither prevented the final fall of body temperature, blood sugar and metabolism, nor prolonged life.

* Research paper No. 204, Journal series, University of Arkansas.

Since the animals in both groups received no food, a specific effect of vitamin B on metabolism certainly could not have been ascertained.

In August of 1929[1] Rose, Stucky, and Cowgill (2) reported that in the majority of dogs studied, anhydremia was found to be a manifestation of a specific influence of a deficiency of the vitamin B complex unrelated to food intake.

Recently Drury, Harris, and Mandsley (3) have reported that deprivation of the vitamin B complex leads to a severe bradycardia in young rats, and that the slow heart rate is due not to the lowered food consumption but directly to the absence of the vitamin.

That vitamin B exerts a specific effect on lactation, unrelated to food intake, has been recently demonstrated by Sure and Walker (4) and by Sure and Smith (5).

In this communication we are reporting our results on the specific effect of vitamin B on growth of the non-lactating weaned albino rat. The first part of the investigation concerned itself with the vitamin B complex and the second part with uncomplicated vitamin B deficiency. About two hundred animals were employed in this study but to conserve space, only representative cases are submitted graphically in Charts 1 to 5 inclusive. The technic employed is essentially that described in the preceding communication and, therefore, needs no repetition here. The work on the vitamin B complex was carried out in pairs, while that on vitamin B was done in triads mainly.

Our findings, as illustrated in the accompanying charts, are self-explanatory and need little comment. An examination of Charts 1 and 2 clearly demonstrates that the vitamin B complex, as supplied by dehydrated Northwestern baker's yeast, produces a specific effect on growth unrelated to the plane of nutrition. Since, however, yeast contains an appreciable amount of foreign substances other than vitamin B, particularly minerals, the question arises whether such inorganic elements as copper (6) and manganese (7), recently reported to be essential in nutrition, may not have been complicating factors. That such is not the case is evident from our studies on uncomplicated vitamin B deficiency in which we used autoclaved yeast and the same yeast in the untreated condition at the same plane of intake. The only limiting factor was vitamin B, which was destroyed in the autoclaved yeast under our conditions of treatment, as is shown in the charted data.

In Charts 3 and 4 a differentiation is made between the specific effect of vitamin B and the higher plane of nutrition on growth.

[1] Published in February, 1930.

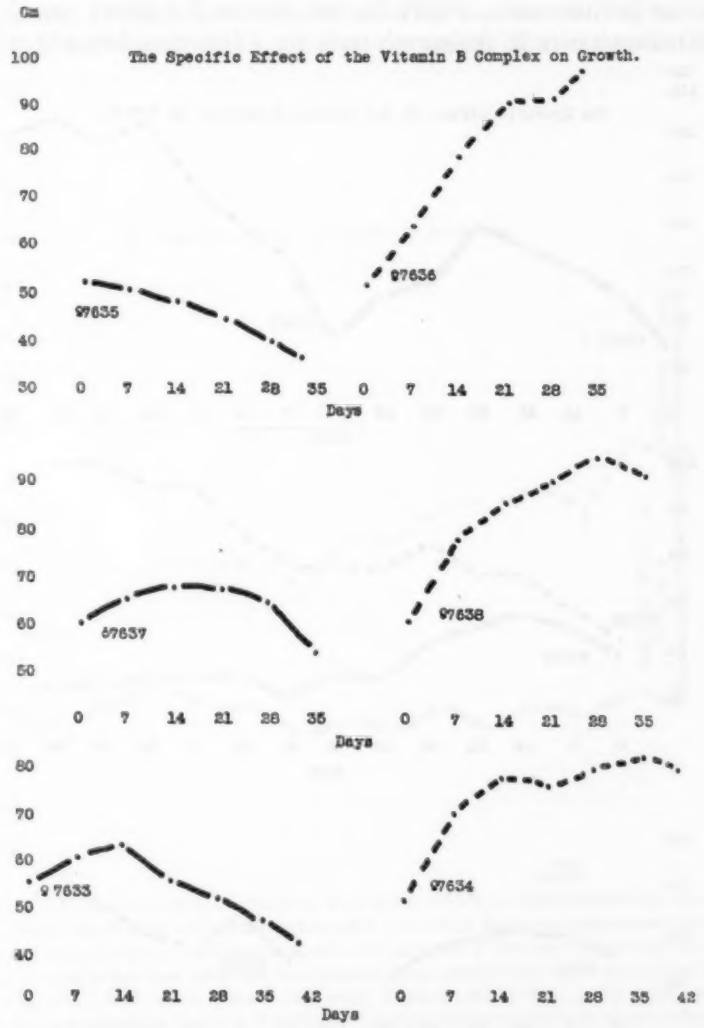

CHART 1. Curves in straight lines represent character of growth of animals on a ration satisfactory in every respect but deficient in the vitamin B complex. Curves in dash lines represent growth records of litter mate rats on the same diet fortified with 10 per cent dried Northwestern yeast as a source of vitamins B and G, and restricted to the same daily food and water intake as their litter mates on the ration deficient in the vitamin B complex.

Since ♀ 7480 (Chart 3) received the same daily amounts of food and water as its litter mate, ♂ 7479 (on the vitamin B-deficient ration) but in addition vitamin B, the growth made by ♀ 7480 must be credited spe-

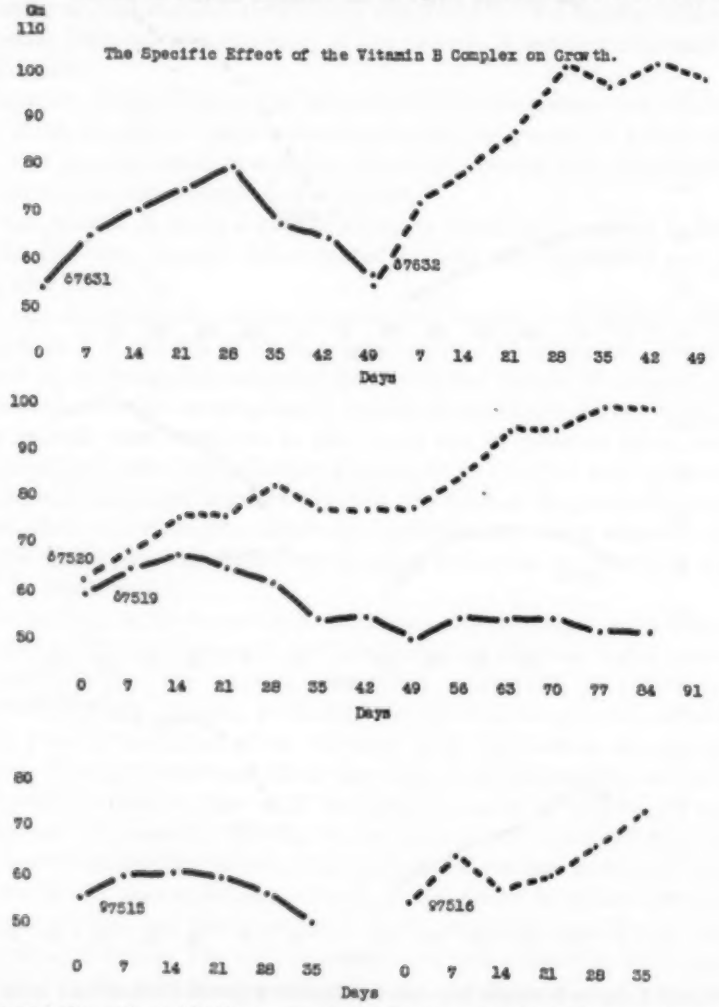

CHART 2. Curves in straight lines represent character of growth of animals on a ration satisfactory in every respect but deficient in the vitamin B complex. Curves in dash lines represent growth records of litter mate rats on the same diet fortified with 10 per cent dried Northwestern yeast as a source of vitamins B and G, and restricted to the same daily food and water intake as their litter mates on the ration deficient in the vitamin B complex.

cifically to this vitamin. The additional increment of growth made by litter mate, ♀ 7482, must be attributed to the fact that its diet was unrestricted, and the difference in the gain of weight between females 7480

CHART 3. Curve 1 represents the character of growth of ♂7479 on a diet deficient only in vitamin B, 10 per cent Northwestern yeast autoclaved for 6 hours at 20 pounds pressure serving as a source of vitamin G; Curve 2 indicates the growth of litter mate, ♀ 7480, on the same diet fortified with 10 per cent of this yeast which has been untreated, and restricted to the same daily food and water intake as ♀ 7479; Curve 3 shows the growth of litter mate, ♀ 7482, on the same diet as ♀ 7480 but unrestricted to its plane of nutrition. A differentiation between the specific effect of vitamin B and the influence of inanition on growth is quite apparent. Similar results are shown in Chart 4.

and 7482 is designated as the inanition effect, since it signifies the growth ♀ 7480 failed to make because it was on a lower plane of nutrition. Similarly, in Chart 4 the difference in gain of weight of females 7445 and 7446

has been produced by the specific influence of vitamin B and the difference in weight between females 7446 and 7482 has been produced by higher plane of nutrition. We must, therefore, now recognize that vitamin B exercises two physiological functions: 1.—it plays a role in metabolism to

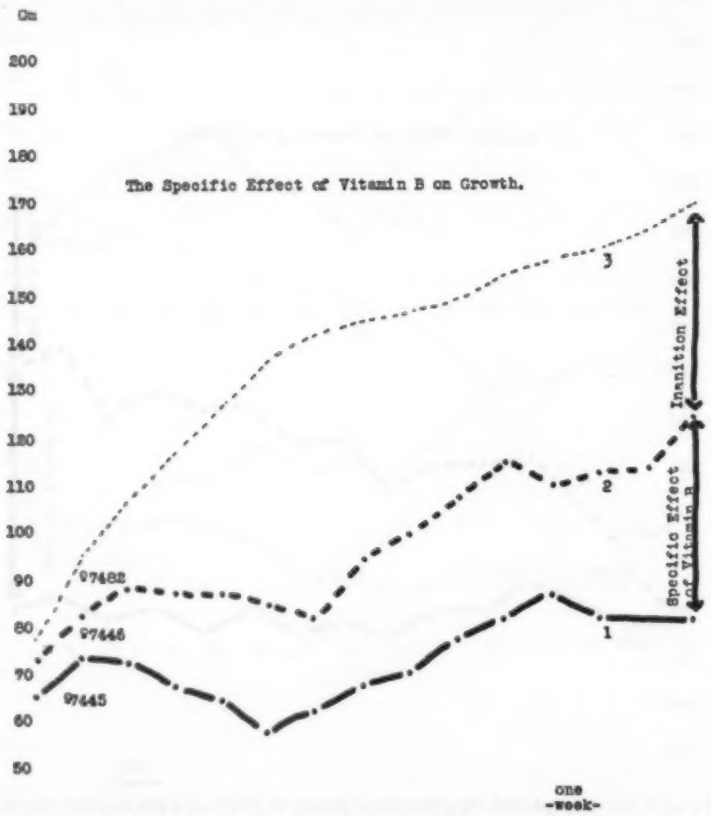

CHART 4. Curve 1 represents the character of growth of ♀ 7445 on a diet deficient only in vitamin B; Curve 2, the growth of litter mate, ♀ 7446, on the same diet fortified with an abundance of vitamin B and restricted to the same plane of nutrition as ♀ 7445; Curve 3, the growth record of ♀ 7482 on the same diet as ♀ 7446 but unrestricted to its daily food and water intake.

the extent that, just as some of the essential amino-acids, it is indispensable for growth without any reference to the food intake; and, 2.—it stimulates the appetite, so as to allow a sufficient intake of all the dietary factors essential for optimum function of all physiological processes in the body.

As little as 0.25 and 0.5 mg. daily of vitamin B concentrate, containing only 0.125 and 0.25 mg. of solids respectively, produced a specific effect on growth. (Chart 5)

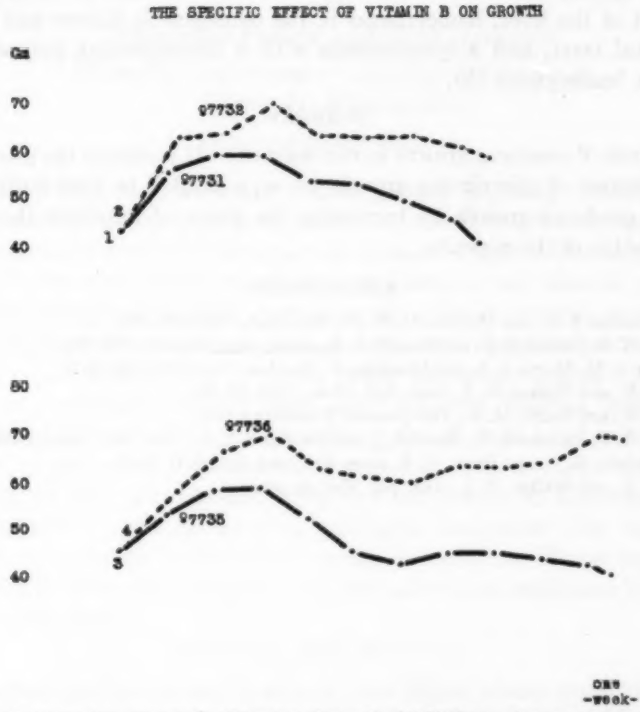

CHART 5. Curve 1 represents the character of growth of ♀ 7731 on a diet satisfactory in every respect but deficient in vitamin B, vitamin G having been supplied in the ration by 10 per cent Northwestern dried yeast autoclaved for 6 hours at 20 pounds pressure, during which process vitamin B was destroyed. Curve 2 shows the growth of litter mate, ♀ 7732, on the same diet and restricted to the same daily food and water intake as ♀ 7731 but ♀ 7732 in addition received daily separately from the ration 0.25 mg. of a highly concentrated vitamin B extract, containing 0.125 mg. solids.

Curve 3 represents the character of growth of ♀ 7735 on a diet deficient only in vitamin B. Curve 4, the growth record of litter mate, ♀ 7736, on the same diet and same plane of nutrition as ♀ 7735, but ♀ 7736 in addition received daily 0.5 mg. of a vitamin B concentrate containing 0.25 mg. solids.

Keeping the plane of nutrition constant, the *specific* effect of vitamin B on growth becomes quite apparent.

The application of our work to the human can be anticipated in borderline diseases associated with malnutrition, i.e., people with a fair appetite

may still be in need of vitamin B for optimum well being, since the exact physiological rôle of vitamin B in the organism is not yet clearly understood. At present we are finding the following pathological conditions as due to specific vitamin B deficiency: Marked reduction in the glycogen content of the liver, hemorrhages in the osteogenetic tissues and in the intestinal tract, and a lymphopenia with a corresponding polymorphonuclear leucocytosis (8).

SUMMARY

Vitamin B produces growth in two ways: 1.—It possesses the physiological function of stimulating growth *per se*, unrelated to food intake; and 2.—It produces growth by increasing the plane of nutrition through a stimulation of the appetite.

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CHANGES IN THE WEIGHTS OF VARIOUS ORGANS AND SYSTEMS OF YOUNG RATS MAINTAINED ON A LOW-PROTEIN DIET

By

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IT IS known that when the normal growth of young animals is prevented by various types of inanition, peculiar dystrophic changes occur, with persistent growth in some parts and loss of weight in others (Jackson, 4, 5, 6). In some earlier experiments of Osborne and Mendel, no such changes (excepting possible growth of the nervous system) were found in young rats maintained for long periods on incomplete protein diets. Mendel and Judson (11), however, noted persistent skeletal growth in mice on inadequate protein diets. More recently Winters, Smith and Mendel (21) observed typical changes in the skeleton, and a few other organs, in young rats with growth retarded by diets deficient in protein, calories, or salts. The present study was undertaken to determine more comprehensively the effects of a prolonged low-protein diet upon the growth of nearly all of the various organs and systems. We are especially indebted to Professor George O. Burr for advice and assistance in regard to the diets used.

MATERIAL AND METHODS

The rats used in this study were of pure Wistar albino stock, reared in the colony at the Institute of Anatomy of the University of Minnesota. A total of 48 rats from 9 litters was used. All were weaned and weighed on the twenty-first day of age, according to the usual routine. Of the 48 rats, 28 were used for the tests and 20 as normal controls, dividing each litter as evenly as possible for this purpose. In the 28 test rats, the average initial weight of the 14 males was 41.14 grams; of the 14 females 40.35 grams; total for both sexes 40.75 grams (range 35 to 48). In the 20 control rats, the average weight of the 10 males was 42.5 grams; of the 10 females 42.8 grams; of the total series 42.65 grams (range 35 to 50). The average weight of the controls was almost the same as the final weight of the test rats (42.42 grams), as shown in Table I.

The 20 control rats after weaning were killed immediately with chloroform. They were then autopsied, and their organs were carefully weighed.

TABLE I

AVERAGE DATA FOR TEST AND CONTROL RATS AT AUTOPSY
WEIGHT IN GRAMS, LENGTHS IN CENTIMETERS

Measurements	Control rats mean (10 m., 10 f.)	Test rats mean (14 m., 14 f.)	Percentage difference in test rats	Significance ratio: Difference / P.E. diff.
Body-weight	42.65 ±0.55	42.42 ±0.41	− 0.5	0.3
Body-length	11.41 ±0.07	12.53 ±0.09	+ 9.8	9.8
Tail-length	9.10 ±0.08	11.204 ±0.095	+23.0	16.9
Organs showing significant increase				
Spinal cord	0.2245±0.0024	0.3575±0.0041	+59.2	27.8
Eyeballs	0.1355±0.0020	0.2085±0.0044	+53.8	15.1
Stomach	0.2985±0.0050	0.4331±0.0080	+44.4	62.9
Skeleton (lig.)	6.677 ±0.035	9.170 ±0.213	+37.3	20.6
Epididymides	0.0336±0.00089	0.0452±0.0019	+34.6	5.5
Hypophysis	0.00163±0.000076	0.00214±0.000046	+31.2	5.7
Liver	2.209 ±0.050	2.569 ±0.039	+16.3	5.6
Head	6.688 ±0.089	7.066 ±0.057	+ 5.6	3.6
Heart	0.2737±0.0033	0.2864±0.0026	+ 4.6	3.0
Organs showing insignificant changes				
Kidneys	0.6881±0.0110	0.7155±0.0073	+ 3.9	2.1
Tongue	0.426 ±0.047	0.435 ±0.038	+ 1.9	0.2
Uterus	0.0327±0.0015	0.0333±0.00077	+ 1.8	0.4
Intestines	0.290 ±0.035	1.306 ±0.020	+ 1.2	0.4
Testes	0.2289±0.0074	0.2312±0.014	+ 1.0	0.1
Brain	1.494 ±0.012	1.501 ±0.0093	+ 0.5	0.5
Lungs	0.3850±0.0082	0.3844±0.0069	− 0.2	0.1
Suprarenals	0.0148±0.00040	0.0137±0.00017	− 7.2	2.4
Pancreas	0.3555±0.0140	0.3185±0.0092	−10.4	2.3
Organs showing significant decrease				
Integument	5.925 ±0.116	5.115 ±0.072	−13.6	5.9
Prostate	0.0295±0.0012	0.0254±0.00035	−13.7	3.2
Musculature	12.955 ±0.225	11.100 ±0.308	−14.3	4.9
Ext. orbital glands	0.0595±0.0016	0.0439±0.0019	−26.0	6.3
Submaxillary glands	0.1794±0.0034	0.1249±0.0034	−30.3	11.2
Ovaries	0.0258±0.0013	0.0136±0.0017	−47.3	5.6
Spleen	0.1460±0.0051	0.0755±0.0016	−48.2	13.1
Thymus	0.1976±0.0068	0.0703±0.0025	−64.4	17.5

In general, the autopsy technic used by Jackson (7) was followed for both control and test animals. In the control rats, the body lengths, tail lengths, and weights of the various organs in most cases were in fairly close agreement with the norms of Donaldson (2) for rats of similar body weight.

The 28 test rats were placed in individual wire cages with wire net floors, allowing the feces to drop through. Body weights were taken daily at the same hour. City tap water was supplied *ad libitum* from bottles with curved tubular glass outlets. The special diet was started immediately after weaning. The basal diet was the following mixture:

	Grams
Sucrose	75.0
Salts (McCollum's mixture 185)	4.5
Lard	20.0
Total	99.5

The basal diet was fed *ad libitum*. The consumption was measured by weighing the initial amount placed in the cage and later the residual amount left in the food cups together with that collected from a paper sheet placed beneath the floor of the cage. The daily average consumption of each rat for the week was divided by his corresponding average body weight to obtain the daily consumption per gram of body weight. Since no sex difference was found, the sexes were combined for the average consumption shown in Fig. 1.

The accessory diet included a mixture of brewer's yeast[1] and dried wheat germ[2] in equal parts, fed in amounts varying from 0.3 to 0.5 gram daily, as shown in Fig. 1. This mixture supplied the vitamin B complex, vitamin E, and also a limited amount of protein. The protein was supplemented, during the 11th week (period of 8 days only), by 0.3 gram of purified casein daily. For vitamins A and D, cod liver oil[3] was administered in dosage of 2 drops daily, increased to 4 drops during the 5th and 6th weeks. All the accessories were mixed and fed separately each day to insure the individual consumption of the amounts indicated.

The experiments extended from May to October, 1929. All of the 28 test rats were kept on the experimental diet at least 15 weeks; 21 of them were continued during the 16th week, and 10 were not killed and autopsied until the 17th week. In Fig. 1, the results are shown only to the 15th week, including all the animals.

In comparing the data for the test and control groups, the probable errors of the means were calculated, using the formulas of Pearl (16). The

[1] Northwestern pure dehydrated.
[2] Washburn-Crosby Co.
[3] Patch.

means were first calculated for the sexes separately, but as no significant difference appeared, the sexes were combined as shown in Table I. The percentage difference for the test rats in each case is found by subtracting the mean for the controls from the mean for the test rats, and expressing this difference (plus or minus) as a percentage of the control mean. In order to determine whether the differences are significant, in each case the absolute difference is divided by the probable error of the difference. The quotient is expressed as the "significance ratio" in the last column of

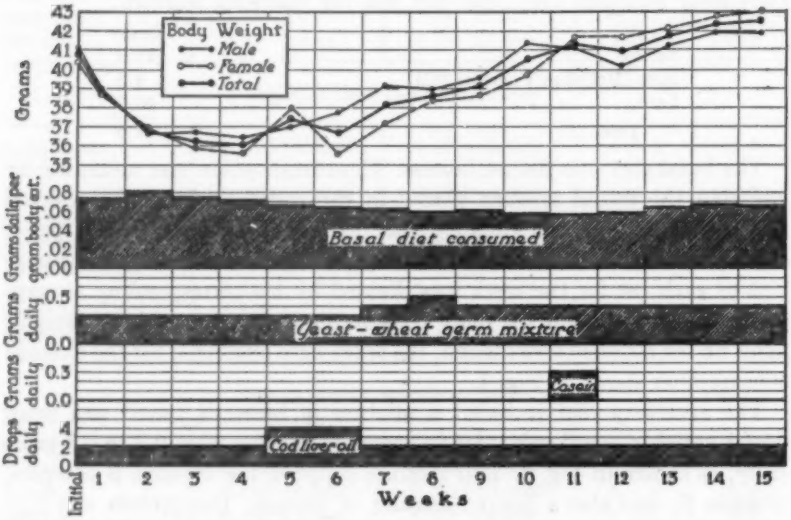

FIG. 1. In the upper part of the figure are curves showing the changes in average body weight for the test rats, male, female and total, for each week up to the fifteenth. The graphs below represent the corresponding intake of the basal diet (grams daily per gram of rat), of the yeast-wheat germ mixture (grams daily), of casein (grams daily, 11th week only), and of cod liver oil (drops daily).

Table I. If this ratio exceeds 3, there is less than one chance in 20 that the difference is due to accidental variation (assuming normal distribution), so the difference is held to be statistically significant.

GENERAL OBSERVATIONS

In general, all of the test rats continued in good health throughout the experimental period of about four months of repressed growth. The external appearance varied somewhat, but there was progressive tendency toward emaciation as the experiment continued. In some cases the rats finally became markedly emaciated, with the characteristic dorsal con-

vexity of the vertebral column. Throughout the experiment most of the rats appeared more than normally active, although there was in some cases a more quiet and drowsy appearance toward the end. The appetite usually remained fairly good, and the daily accessories (yeast, wheat germ and cod liver oil) were consumed greedily when placed in the cages. The hair coat and eyes remained normal. No evidences of vitamin deficiency (ophthalmia, rickets, polyneuritis, pellagra) were noted during the course of the experiment or at autopsy.

Body Weight and Dietary Relations

Body weight. The curves of body weight (Fig. 1) show no consistent sex difference, so that in the following discussion the average will be taken for the sexes combined. It is evident that the mean body weight declined steadily from 40.75 grams at the beginning to about 36 grams during the 3rd and 4th weeks. This corresponds to an average loss of nearly 12 per cent, the individual losses ranging from 8 to 25 per cent of the initial weight. Thereafter there was a slow but fairly steady gain in average weight. In the 11th week it slightly surpassed the initial weight, averaging 41.3 grams. It decreased slightly to 40.9 grams in the 12th week, but increased thereafter to 41.7 grams in the 13th week, 42.3 grams in the 14th and 42.4 grams in the 15th week. The final average for the test rats (some continued into the 16th and 17th weeks) was 42.42 grams, as given in Table I. The final individual weights ranged from 35 to 46 grams in the test rats, and from 35 to 50 grams in the normal controls.

Dietary relations. In relation to the changes in body weight of the test rats, the adequacy of the total food intake (caloric value) as well as of the fat, salts, protein and vitamins should be considered. The water intake was not measured, but may be assumed to be adequate, as it was supplied *ad libitum*. Unfortunately the same assumption cannot be made for the total consumption of the basal diet similarly supplied *ad libitum*, since it is known that the voluntary food intake may decrease when necessary factors are lacking.

In the present experiments, as shown in Fig. 1, the voluntary daily consumption of the basal diet averaged 73 milligrams per gram of rat during the first week, increasing to 82 milligrams during the 2nd week. Thereafter, it slowly decreased to a minimum of 57 milligrams in the 11th week, rising again to about 66 milligrams in the 14th and 15th weeks. If we add the weight of the yeast-wheat germ mixture and of the casein (11th week only), the daily total food intake (aside from the cod liver oil) per gram rat becomes 81 milligrams in the first week, 90 in the 2nd,

82 in the 3rd, decreasing to 68 milligrams in the 10th week. With the casein in the 11th week the daily intake per gram rat rises to 74 milligrams, followed by 68, 74, 77, and 75 milligrams in the 12th to the 15th weeks, respectively.

The average daily intake of basal diet in grams per rat for the 15 consecutive weeks (in order) was 2.85, 3.01, 2.68, 2.56, 2.52, 2.35, 2.34, 2.33, 2.37, 2.34, 2.34, 2.39, 2.68, 2.83, 2.78.

It seems unlikely that the decline in body weight during the first three weeks was due to inadequate energy intake, as the consumption per gram of rat at this time (even without the cod liver oil) was equal to or greater than that found by Osborne and Mendel (12) to be adequate for maintenance in rats of similar size (30 to 50 grams) on diets of similar caloric value. Wang, Huddlestun, and Saphir (20) obtained some growth for 20 weeks with a food intake of only 58 milligrams per gram rat per day, but the exact composition of this food is not stated. The adequacy of the energy intake in our tests would appear to be evidenced also by the slight but continued growth later upon a slightly decreased relative food intake. But in this connection, as observed by Jackson (4) and others, it must be remembered that the food intake required for maintenance in growing animals decreases notably as the experiment continues, probably on account of lowered basal metabolism.

The necessary fat (as shown by Burr's work) was provided by the lard in the basal diet. For the adequacy of the salt intake we have no definite evidence, beyond the fact that the proportion used in the basal diet has been found amply sufficient for growing animals with larger intake of adequate diets. The actual amount of the salt mixture required for maintenance is not definitely known, but Osborne and Mendel (13) and Winters, Smith, and Mendel (21) have shown that growth in rats can be suppressed or retarded by low salt diets.

The protein content of the diet, excepting the 11th week, was supplied solely by the yeast-wheat germ mixture. According to the analyses cited by Osborne and Mendel (14) and Horwath (3), the actual protein content will scarcely exceed one-third in the case of either yeast or wheat germ. According to this estimate the protein content for 0.3 gram of the mixture would be 100 milligrams. On this basis, the protein intake per gram rat per day during the 15 weeks (in order) averaged as follows in milligrams: 2.6, 2.7, 2.8, 2.8, 2.7, 2.7, 3.5, 4.3, 3.4, 3.3, 10.5, 3.3, 3.2, 3.2, 3.2. Excepting the 11th week (when the casein was added), this is far below the minimum casein intake (about 12 milligrams daily per gram rat) recorded by Osborne and Mendel (12) in rats maintained at similar weight (30 to 50

grams). Wang, Huddlestun, and Saphir (20) found 4 milligrams of casein daily per gram rat to be sufficient protein for slow growth in rats of 50 to 60 grams, but the casein was apparently "supplemented by a definite amount of yeast and lettuce," the protein content of which is not stated. With only 2 milligrams of casein, their rats lost weight. Although our rats lost weight during the first two or three weeks, the protein intake was sufficient thereafter to permit maintenance and even a slight increase in weight, as is apparent in Fig. 1.

The increase in body weight of our rats on such low protein intake is somewhat difficult to explain, unless we assume that the proteins of the yeast-wheat germ mixture are superior to casein. Osborne and Mendel (14) found the proteins of the wheat germ more efficient than those of the entire kernel in promoting growth of rats. Osborne and Mendel (15) and others have kept growing rats over long periods on diets in which yeast furnished the sole source of nitrogen as well as of water-soluble vitamin. This shows that yeast protein (in sufficient quantity) is adequate for growth. Still and Koch (19), however, observed that the growth of young rats on diets containing yeast as the sole source of the protein is less satisfactory than that when the yeast is replaced by casein. Nevertheless they found no special amino acid deficiency in the yeast protein. If protein was the limiting factor retarding growth in our rats, it is surprising that the addition of casein had so little effect in the 11th week, but possibly the time allowed was insufficient.

Finally the adequacy of the vitamin content of the diet must be considered. Two drops of cod liver oil[4] daily was assumed to supply vitamins A and D in amounts adequate for slight growth at this level, in accordance with the experience of Burr.[5] Moveover, slight growth on this dosage did occur in the latter half of the experiment. The slight, apparent response when the oil was increased to four drops in the 5th week is therefore probably insignificant, especially as the body weight decreased slightly in the following week on the same dosage (Fig. 1). Similarly Burr has found the 0.3 gram yeast-wheat germ mixture daily ample to supply the vitamin B complex and vitamin E for moderate growth. It is therefore improbable that the slight improvement in growth from the 7th week onward was due to the increase in these factors with the increased amount of the yeast and wheat germ. On the whole, it appears more probable that the limiting factor was rather in the protein content. However, it must be frankly ad-

[4] Patch.

[5] Personal communication.

mitted that other deficiencies may have existed. Further work would be necessary to settle this question definitely.

Organ Weights

In Table I are given the mean organ weights for the test rats and controls, the percentage change in the test rats, and the significance of this change as indicated by the significance ratio. For convenience, the organs of the test rats are grouped under those showing 1.— a significant increase; 2.— insignificant change; and 3.— a significant decrease.

Organs showing significant increase. These include, in decreasing order, the spinal cord, eyeballs, stomach, ligamentous skeleton, epididymides, hypophysis, liver, head and heart. The increases in weight (estimated by comparison with the weights in the normal controls of the same body weight) range from 59.2 per cent in the spinal cord down to 4.6 per cent in the heart. The marked increase of 37.3 per cent in skeletal weight is in accordance with the increase of 9.8 per cent in body-length, measured from nose to anus. That this persistent skeletal growth varies in different regions of the body is indicated by the greater relative increase of 23 per cent in the tail-length.

Organs showing insignificant changes. As indicated in Table I, the organs showing but slight changes in weight are the kidneys, tongue, uterus, intestines, testes, brain, lungs, suprarenals, and pancreas. The increase of 3.9 per cent in the kidneys, and the losses of 7.2 and 10.4 per cent, respectively, in suprarenals and pancreas, might be considered as probably (but not certainly) significant, since the significance ratio in these cases is between two and three.

Organs showing significant decrease. The organs in this group, in order of increasing relative losses in weight, are the integument, prostate, musculature, external orbital glands, submaxillary glands, ovaries, spleen and thymus. The losses range from 13.6 per cent in the integument to 64.4 per cent in the thymus.

Discussion

The results of the present study may be compared with those of previous investigations, including those in which growth of rats was repressed or retarded by dietary deficiencies of protein, calories, water, salts, or vitamin D (rickets).

The effects of protein deficiency on the weights of a few organs were observed by Winters, Smith, and Mendel (21). In young rats maintained several weeks on an inadequate protein diet, they found an average increase of about 4 per cent in body length, with a corresponding increase of

50 to 60 per cent in the weight of the skeleton (leg bones). Increases (usually slight) were noted also in the brain, liver, heart and lungs, and especially in the kidneys (55 per cent). Wang, Huddlestun, and Saphir (20) found that on very low protein diets, rats at 50 to 60 grams body weight became markedly emaciated and inactive, mostly dying at the 16th to 22nd week. At somewhat higher protein level, the body weight doubled in this time. At all levels of protein intake the kidneys remained normal in weight, in agreement with our results for low protein diet but in contrast with those of Winters, Smith, and Mendel.

Jackson (4) held rats at about 24 grams in body weight by general underfeeding from 3 to 10 weeks of age. The average changes in body length and organ weights were remarkably similar to those found in the present study. Marked increases occurred in the eyeballs, spinal cord, testes, skeleton stomach-intestines, suprarenal glands, hypophysis, and liver. Slight changes (below 10 per cent) were noted in the head, kidneys, musculature, brain, and heart. Decreases were found in the lungs, ovaries, skin, spleen, and thymus. As compared with the present results on low-protein diet, the main differences were in the testes and suprarenals, showing marked increases of 34 and 26 per cent, respectively; and in the skin, with a loss of 36 per cent. Winters, Smith, and Mendel (21) in young rats maintained on low-calorie diet similarly found skeletal growth, with increase of 4 per cent in body length and of 50 to 60 per cent in weight of leg bones. The brain showed slight increase, but the kidneys increased 55 per cent. The other organs were not mentioned. These results coincide with those found by them in low-protein tests.

Kudo (10) similarly maintained rats averaging 27 grams over a period of 13 weeks by low-water diet. The changes in organ weights were in general similar to those found in the present study, but there are some exceptions. The testes decreased 49 per cent in weight, the musculature only 4 per cent. On the other hand, the kidneys increased 58 per cent and the suprarenals 68 per cent. Jackson and Smith (9) more recently retarded rats of 50 to 70 grams by deficient water intake for longer periods of 16 to 33 weeks. The changes in organ weights resembled those found by Kudo, but the testes increased 70 per cent while the liver lost 30 per cent.

Comparison may also be made with the results of Jackson and Carleton (8) in rats 4 to 8 weeks old, body weight 30 to 75 grams, stunted in growth by a rachitic diet. The skeleton, in spite of the rachitic changes, averaged 15 per cent above normal weight. The eyeballs, suprarenals, kidneys, lungs, heart, ovaries, and submaxillary glands were also above normal. The head, testes, stomach-intestines, liver, spleen, and lungs were nearly

normal, while the hypophysis, musculature, skin, and thymus were definitely subnormal in weight. The changes in organ weights are in part similar to those found in protein deficiency, but there are several obvious differences.

In rats retarded by low-salt diets, Winters, Smith, and Mendel (21) observed an increase of 3 per cent in average body length, in spite of a loss of 30 to 40 per cent in skeletal weight (leg bones). A slight increase in brain weight and a marked gain of 55 per cent in the kidneys duplicated their findings with inadequate protein and low calorie diets. In similar tests with low-salt diets, Smith and Schultz (18) likewise noted increase in body and tail lengths, and in skeletal weight, with striking enlargement of the kidneys. The spleen decreased in weight.

In general, the foregoing results indicate clearly that during repression of growth in body weight by various types of dietary deficiency, there are striking dystrophic changes among the individual organs, some increasing markedly, while others undergo atrophy (Jackson, 5, 6) (Smith, 17). When the results in various dietary deficiencies are compared, it is evident that the changes in organ weights in many cases are remarkably similar. The similarities in organ changes under varied types of inanition may be due to a common underlying factor—interference with the general process of nutrition. This interference is generally associated with a reduced food intake, which may overshadow the more specific effects of deficiencies in the individual nutritional elements concerned.

The differences in organ changes recorded under various nutritional deficiencies may be in part ascribed to accidental variations, or to differences in the age or strain of the animals, length of the experiment, etc. In part, however, the differences in organ response are probably due to more specific failure to supply the nutritional requirements of these organs. Such nutritional requirements differ considerably in different organs, and even in the same organ at different ages and stages of development. Nutritional deficiences can therefore upset the normal growth process by permitting some organs to continue growth while others remain stationary or undergo atrophic changes.

Summary

1. Twenty-eight rats were fed from weaning time (age 3 weeks) a diet low in protein but planned to be adequate in calories and in the various accessories. The rats remained healthy and active over a period of about 4 months. No sex difference or evidence of specific nutritional disorder was apparent. The average body weight decreased from about 41 grams to 36

grams by the end of the first month, thereafter increasing slowly to about 42 grams at the end of the test.

2. Finally the test rats were killed and autopsied, and likewise 20 normal control rats of the same body weight for comparison. The average body length of the test rats had increased about 10 per cent, and the tail length 23 per cent, in comparison with the controls.

3. The average organ weights are considered in 3 groups. Those showing a significant increase in weight in the test group are (in order from greatest to least change): spinal cord, eyeballs, stomach, skeleton, epididymides, hypophysis, liver, head, and heart. The changes vary from about 59 per cent in the spinal cord to 5 per cent in the heart.

4. The organs showing but slight (statistically insignificant) change are the kidneys, tongue, uterus, intestines, testes, brain, lungs, suprarenals, and pancreas.

5. The organs showing significant decrease in weight are (in order) the integument, prostate, musculature, external orbital, and submaxillary glands, ovaries, spleen, and thymus. The losses vary from about 14 per cent in the integument to 64 per cent in the thymus.

6. The dystrophic changes in organ weights in many respects show a remarkable degree of correspondence in various types of nutritional deficiency. This may be due to the common underlying factor of interference with the general process of nutrition, usually associated with a deficient intake of food. On the other hand, differences in the changes among the various organs (comparing different organs during the same nutritional deficiency, or the same organ during different deficiencies) may be due to specific differences in their nutritional requirements.

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FAILURES TO PRODUCE EXPERIMENTAL DENTAL CARIES IN THE WHITE RAT WITH HIGH CARBOHYDRATE DIET AND BACILLUS ACIDOPHILUS OR WITH VITAMIN D DEFICIENCY

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NO PROBLEM is of greater interest to humanity today than is that of dental caries. And no problem is older. Three thousand years B.C. the Egyptians were using a preparation of flint, leaves, and honey to prevent caries. At the same time the Chinese were using musk and salt for the same purpose. Aristotle thought sweet, soft figs decayed the teeth. Hippocrates held "humors of the body" responsible for caries. Pliny recommended cleaning the teeth to prevent their decay. The Romans advocated the use of tooth picks and dentifrices, as did the Arabians. In the middle ages caries was attributed to a "worm in the tooth." In 1728 horse hair tooth brushes were condemned as harmful by the French. Thomas Berdmon, dentist to King George III, advocated soft tooth powder to clean the teeth and for the prevention of caries. Westcott, in 1843, showed that acids originated from fermentation and thought that such acids dissolved the enamel and produced caries. Harris, in 1853, and Tomes, in 1873, ascribed caries to acids produced by fermentations.

Miller (1) advanced his important parasite theory, and claimed that organisms in the mouth act on carbohydrate materials and by a process of fermentation form lactic, butyric, acetic, and other acids which destroy the enamel. McIntosh, James, and Lazarus-Barlow (2) postulated a constantly present bacillus capable of producing acids by fermenting carbohydrate material, and reported that this bacillus resembled the acidophilus group of Moro. Bunting (3) reported a series of studies in which he believed he had correlated the formation of dental caries with the activities of the Bacillus acidophilus. Later, he (4) published further studies to the effect that certain individuals appear to have an immunity to the Bacillus acidophilus, but that this immunity may disappear over varying periods of time, after which caries may ensue. McCollum (5) several years ago called attention to the importance of scientific nutrition, during the period of growth, as well as to the proper nutrition of the expectant and nursing mother in order to develop sound teeth in the offspring.

Other observers have attributed the etiology of dental caries to a deficiency of vitamin C. The outstanding exponents of vitamin C deficiency in relation to caries are Zilva and Wells (6), who reported changes in the teeth of guinea pigs that had been fed a scorbutic diet. Höjer (7) produced dental lesions in guinea pigs by the use of vitamin C-deficient diets. P. R. Howe (8) reports the production of dental caries in monkeys fed a scorbutic diet. Hanke (9) accepts the findings of Howe and Höjer.

May Mellanby (10, 11, 12) has extensively studied the relationship of vitamin D to the calcification of teeth in puppies, and concludes that a deficiency of vitamin D results in the production of imperfect (hypoplastic) teeth, which are subject to early decay.

It has now been pointed out that some workers attribute caries to the action of an organism; others believe it to be due to a deficiency of vitamin D; and still others to a deficiency of vitamin C. The first two hypotheses have not received general acceptance. Thus, McIntosh, James, and Lazarus-Barlow (13) failed to produce caries in rabbits and monkeys with Bacillus acidophilus. Rosebury and Karshan (14) studied the relationship of diet to dental caries. Their experiments were conducted over periods of forty to sixty days, and in these short periods they were unable to produce caries. Their results were as follows:

> Sixty young rats were studied in three groups, in which were tested, respectively, a low-calcium, vitamin D-free diet, and a diet high in fermentable carbohydrate (all synthetic), in each case with and without the addition of human oral Lactobacilli fed in an adhesive paste. All rat mouths were cultured at intervals for aciduric organisms. The character of the growth obtained with the different diets is recorded. The presence of rickets in the first group was ascertained by line tests and ash determinations on the tibias. Aciduric bacteria similar to *Lactobacillus acidophilus* were found to be a normal inhabitant of the rat mouth, their presence being constant and independent of the feeding of human Lactobacilli. No dental caries was produced, in spite of evidence of retention of food in molar pits, probably indicating the absence in these experiments of one or more factors in susceptibility to caries.

We regarded the white rat as a poor animal in which to study vitamin C deficiency on account of its apparent ability to survive normally on a diet deficient in this vitamin. We therefore elected to study the other two possible postnatal factors, namely, a high carbohydrate diet and Bacillus acidophilus, and a vitamin D deficiency.

Because of the lack of agreement among investigators regarding the relationships of the various factors to caries, it seemed important to reexamine the question with the full knowledge that much of the confusion is attributable to the brevity of the feeding experiments. The decisive work of the past few years in the study of chronic diseases has emphasized the point that success depends at least in part on prolonged trials of any dietary factors.

Since some observers have attributed dental caries of offspring to the faulty nutritional condition of the mother during gestation and nursing, while others emphasize dietary abnormalities in effect after weaning, it was desirable to separate the undertaking into two divisions. For the present we chose to study, over long periods of time, the problem in the white rat after weaning. In order to avoid harmful conditions before this age had been reached, the experimental animals were selected according to the following plan: All animals used were at least the fifth generation of an inbred stock that had been fed a diet, *ad libitum*, consisting of fresh meat, whole milk, bones, bread, and fresh and cooked vegetables. The success of this diet is evidenced by the large size of the young at weaning, since when thirty days old the rats weighed from 55 to 70 grams each, depending on sex. The animals were kept in a large, well ventilated and well lighted room in wire cages, which were cleaned daily. With these animals, thus produced, two major questions were studied.

Series I

The animals of this series were placed on the experimental diet when thirty days old. Six groups of animals were employed. Group 1 (12 rats) received a diet originally devised by Mendel and used subsequently with satisfaction by many other observers as a normal diet for the growth and maintenance of the white rat. It consisted of:

	per cent
Starch	53
Lard	25
Casein	18
Mendel's normal salt mixture	4

With this, 0.03 per cent by weight of Viosterol (potency 100 D) was incorporated. In addition each rat received daily 10 grams of fresh green cabbage, 1 gram dried brewer's yeast, and tap water *ad libitum*.

Group 2, (12 rats). These animals received the same diet as group 1, with the exception that the starch of the Mendel diet was replaced by sucrose. Both groups were infected *per orum* with a pure culture of Bacillus acidophilus at the beginning of the test. The rats were kept on this diet for nine months, at which time they were killed and examined for dental caries. No caries was found.

In the next four groups, cabbage was replaced by lettuce. Otherwise groups 3 and 4 received the same diet as group 1, while groups 5 and 6 received the same diet as group 2.

In addition each rat in group 3 (starch) and in group 5 (sucrose) had about 1 cc. of a pure culture of Bacillus acidophilus forced into the mouth and rubbed over the teeth and gums with a large wire loop three times a week throughout the entire period of the feeding. There were 7 rats in each of these four groups. These 4 groups were fed the diet for one year (or until the animals were 13 months old), and were then killed and examined for

FIG. 1. A typical section of a mandibular molar of a rat that received a high carbohydrate diet and tri-weekly inoculations of Bacillus acidophilus. The dental tissue is entirely normal. On gross inspection a possible lesion was suspected, but the microscopic examination showed that the suspicious area was merely foreign material in an occlusal pit.

caries. After a careful search of all the teeth of all the rats of these groups, no single example of caries was found.

SERIES II

Steenbock's rachitogenic diet No. 2965 was fed to ten rats that were kept in individual wire cages in a dark room. Four of these rats received 0.03 per cent Viosterol (potency 100 D), which was thoroughly incorporated in the diet. One rat died in 6½ months, the remaining three were killed after one year of feeding, or when they were thirteen months old. No

x-ray evidence of rickets, bone or joint deformities was discovered in this group.

The remaining six rats, that received Steenbock's rachitogenic diet only, exhibited the usual appearances produced by a rachitogenic diet. One rat lived 3 months, four others lived 4, $5\frac{1}{2}$, $8\frac{1}{2}$ and 11 months respectively and the sixth rat was killed after one year of feeding, or when

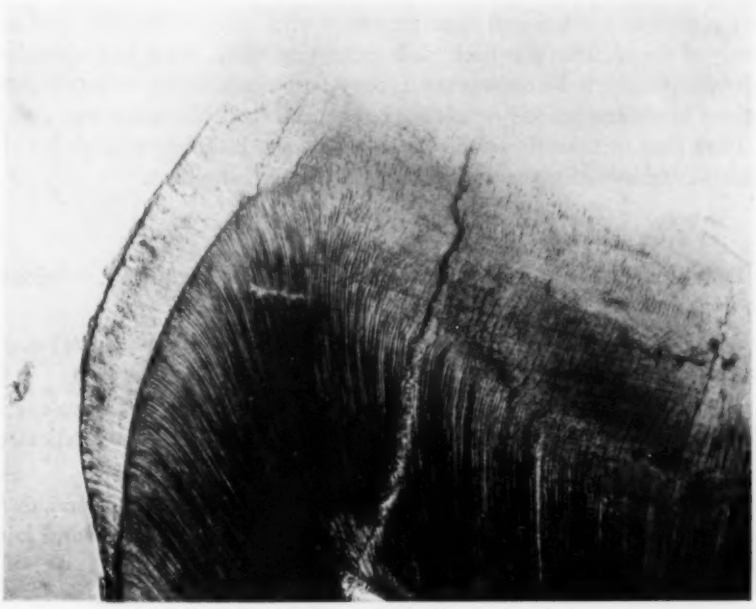

FIG. 2. A similar preparation from a rat that received a rachitogenic diet in the absence of vitamin D for one year. The rat was kept in a dark room throughout the feeding period. The tooth is entirely normal.

thirteen months old. All six of these animals showed x-ray evidence of florid rickets, with marked bone and joint deformities. The teeth in all the rats of this series were examined for caries and none found.

Preparation of the teeth

The superior and inferior maxillae were removed, the soft tissues dissected away and cleaned with water, a tooth brush and tooth powder. The teeth under illumination and magnification were then scrutinized for defects. All teeth showing the slightest suggestion of carious lesions were ground longitudinally by means of a dental engine and a fine carborundum

wheel to a plane bisecting the center of what appeared to be the carious lesion. This ground surface was then polished smooth by means of a crocus disc. The teeth were then imbedded on a glass slide by means of water-soluble glass, the polished surface toward the slide. The reverse surface was then ground down by means of a fine sandpaper disc until an approximate thickness of fifty micra was attained. This surface was then polished with a crocus disc. The section was then removed with water and placed in alcohol and then mounted with balsam without staining. Study of the sections was then made microscopically. What had appeared, microscopically, to be carious areas, proved on examination to be accumulations of an amorphous debris in the occlusal pits. No caries was found.

I am glad to take this occasion to thank Dr. L. H. Newburgh for the interest and advice given me throughout this investigation.

Conclusion

1. White rats, fed for one year on a normal diet (Mendel's), developed no dental caries.

2. White rats, fed for one year on a diet 53 per cent of which was sugar (sucrose), but otherwise normal, developed no dental caries.

3. White rats fed for one year on a diet 53 per cent of which was sugar, but otherwise normal, and which received, triweekly, oral inoculations of a pure culture of Bacillus acidophilus, developed no dental caries.

4. White rats which survived on a rachitogenic diet for long periods (8 months to one year) developed florid rickets with extreme bone and joint deformities, but no dental caries.

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THE ANALYSIS OF THE CURVE OF HEAT PRODUCTION IN RELATION TO THE PLANE OF NUTRITION

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Introduction

IN RECENT studies at the Pennsylvania Institute of Animal Nutrition (1,2) the energy metabolism of cattle was studied at seven planes of nutrition, namely, 1.–fasting, 2.–half of the maintenance requirement, 3.–maintenance, 4.–half more than maintenance, 5.–two times maintenance, 6.–two and a half times maintenance, and 7.–three times maintenance; and was described in the second of the papers cited in language as follows (2, p. 77):

> With the heat production of the fourth day of inanition as the base value, the heat production increased slowly between fasting and maintenance, and much more rapidly above maintenance, but with a decreased rate of rise between the planes of twice and three times maintenance.
>
> The curve of heat production in relation to the plane of nutrition was found, therefore, to be a reversed or S curve.
>
> The heat production of fasting being considered as including two factors, a waste heat of utilization of body nutrients katabolized, and a theoretical minimum base value, including no such waste—the curvature of the line of heat production in relation to increasing food consumption is interpreted as resulting from: 1.—the increasing concentration of metabolites circulating in the blood; 2.—the change in the proportions of protein, fat, and carbohydrate katabolized, with increase in the katabolism of food nutrients and decrease in the katabolism of body nutrients; 3.—the energy expense of synthesis of body nutrients (fat from carbohydrates); and 4.—the decreased metabolizability of the food at the higher planes of nutrition.

The idea of a hypothetical minimum heat production, less than the heat production of fasting, is suggested by the character of the curve of heat production as viewed from its upper end. From this point of view, as the katabolism of body substance begins to contribute a part of the heat, the direction of curvature is reversed and the degree of curvature is prominently and progressively increased—thus revealing the influence of a non-rectilinear component—the specific dynamic action of body substance katabolized.

These curves of heat production are presented in Figs. 1 and 2, and the data on which they are based are given in the first and the last columns of Table I.

In the papers referred to it was recognized that on account of the progressive replacement of food nutrients by body nutrients between main-

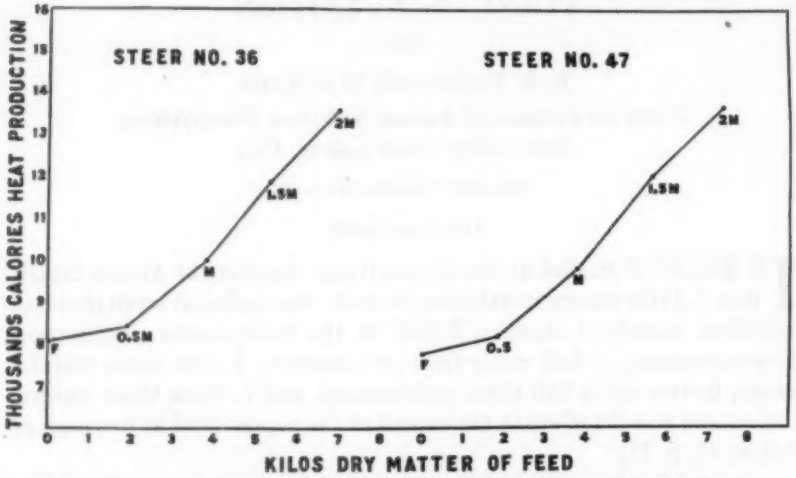

FIG. 1. The curve of heat production of cattle in relation to the quantity of feed eaten.

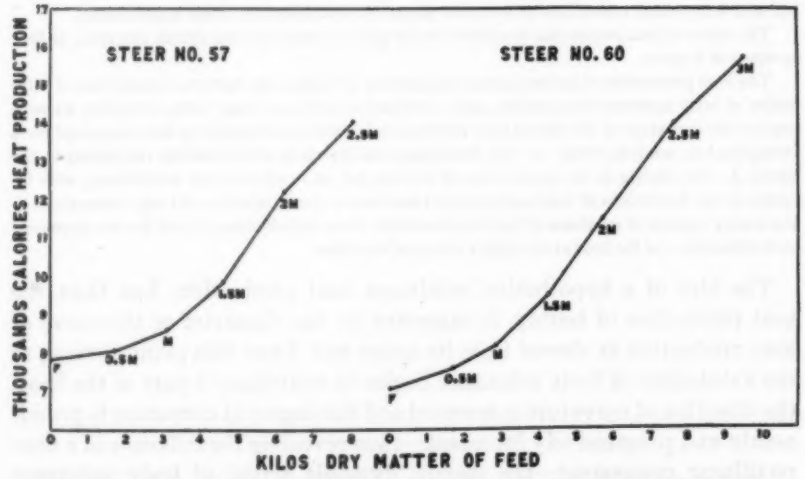

FIG. 2. The curve of heat production of cattle in relation to the quantity of feed eaten.

tenance and fasting, the heat increments at the lower planes of nutrition, as related to the food, were less than the true energy expense of food utilization by the amount of the decrement of heat of dynamic action of body

nutrients katabolized between the same planes of nutrition; and that the curvature of the line representing the relation of the heat production to the food consumption between fasting and maintenance is determined by the excess of the waste heat of utilization of increasing food nutrients as compared with that of decreasing body nutrients katabolized.

The break in the curve at the maintenance point may be due to a greater specific dynamic effect of food carbohydrates than of body fat, in cattle; and the reversed curvature at the higher points of observation appears to be due mainly to diminished metabolizability of the ration.

It is true that it is illogical, in a sense, to relate the heat production to a source (food nutrients) which produces all of it at maintenance and above, and none of it at fasting; but the practical interest in the food as the usual source of heat justifies this procedure. The more logical reference of the heat production to the energy of the nutrients katabolized, regardless of source, yields a curve which differs from the one discussed above only between fasting and maintenance as a result of the shortening of the abscissas—having the effect to increase the rate of rise in the curve between these two points, though this rate of rise remains less than that which prevails above maintenance.

The best support of the validity of these curves derives from the fact that whereas in experiment No. 238 (steers No. 36 and 47) the sequence of the experimental periods, as indicated by the plane of nutrition, was 2.0 maintenance, 1.5 maintenance, 0.5 maintenance, 1.0 maintenance, and fasting, in experiment No. 240 (steers No. 57 and 60) the sequence was radically changed, to the following order—1.0 maintenance, 1.5 maintenance, 2.0 maintenance, 2.5 maintenance, 3.0 maintenance, 0.5 maintenance, and fasting.

In order to make certain that the measurements of the fasting heat production in the two experiments might not possibly be affected by previous feeding at different planes of nutrition, the fasting periods in experiment No. 240 were preceded by preliminary feeding at a plane of maintenance, during which no heat measurements were made.

It is inconceivable that the curves representing the two experiments could have been so closely similar, under the circumstances, if the heat production, as determined, had not been characterized by a very nearly uniformly high degree of validity as representing the quantities of food consumed.

A research conducted by Gigon (3), contributes to the understanding of the relation of heat production to plane of nutrition. In a series of experiments he measured the energy metabolism of a man when fed casein ex-

TABLE I

PARTITION OF THE ENERGY OF THE AVERAGE DAILY FEED AND THE HEAT PRODUCTION OF CATTLE AT DIFFERENT PLANES OF NUTRITION

Experiment, animal and period No.	Plane of nutrition	Dry matter of feed	Gross energy	Energy of feces	Energy of urine	Energy of methane	Digestible energy	Metabolizable energy	Heat increments	Heat production*
		Kgm.	Cals.	Cals.	Cals.	Cals.	Cals.	Cals.	Cals.	Cals.
Exp. 238										
Steer 36										
Period 12	Fasting	—	—	—	—	—	—	—	—	8,060
" 6	½ maintenance	1.885	8,517	2,327	463	831	6,190	4,896	374	8,434
" 8	Maintenance	3.762	16,997	4,466	802	1,481	12,531	10,248	1904	9,964
" 4	1½ times maintenance	5.353	24,189	6,669	1,043	1,842	17,520	14,636	3787	11,847
" 2	2 times maintenance	7.037	31,799	9,794	1,217	2,168	22,005	18,620	5554	13,614
Steer 47										
Period 11	Fasting	—	—	—	—	—	—	—	—	7,790
" 5	½ maintenance	1.863	8,417	2,262	432	827	6,155	4,895	399	8,189
" 7	Maintenance	3.790	17,124	4,399	768	1,449	12,725	10,508	2013	9,803
" 3	1½ times maintenance	5.617	25,382	6,940	1,047	1,880	18,442	15,515	4214	12,004
" 1	2 times maintenance	7.384	33,367	10,142	1,255	2,445	23,225	19,525	5921	13,711
Exp. 240										
Steer 57										
Period 14	Fasting	—	—	—	—	—	—	—	—	7,838
" 10	½ maintenance	1.700	7,596	2,072	387	745	5,524	4,392	223	8,061
" 2	Maintenance	3.085	13,778	3,629	623	1,272	10,139	8,243	760	8,598
" 4	1½ times maintenance	4.612	20,608	5,484	874	1,781	15,124	12,469	2049	9,887
" 6	2 times maintenance	6.233	27,851	8,029	1,123	2,335	19,822	16,363	4377	12,215
" 8	2½ times maintenance	8.057	36,026	10,880	1,360	2,930	25,146	20,855	6200	14,038

* Corrected to represent the standard day and uniform live weight.

Steer 60										
Period 15	Fasting	—	—	—	—	—	—	—	—	7,034
" 11	½ maintenance	1.681	7,511	2,128	374	738	5,383	4,270	559	7,593
" 1	Maintenance	2.828	12,630	3,461	562	1,061	9,169	7,545	1161	8,195
" 3	1½ times maintenance	4.237	18,933	5,466	743	1,483	13,467	11,241	2466	9,500
" 5	2 times maintenance	5.704	25,488	7,484	1,017	2,086	18,004	14,902	4504	11,538
" 7	2½ times maintenance	7.520	33,602	10,337	1,280	2,518	23,265	19,467	6932	13,966
" 9	3 times maintenance	9.489	42,429	13,959	1,500	3,075	28,470	23,896	8666	15,700

* Corrected to represent the standard day and uniform live weight.

clusively in quantities of 50, 100, 150, and 200 grams, and measured also the basal metabolism of his subject. He found that the increase in heat production above the basal value was 19 calories, or 7.7 per cent, on the 50 gm. dose; 53 calories, or 13.1 per cent, on the 100 gm. dose; 118 calories, or 24.0 per cent, on the 150 gm. dose; and 171 calories, or 25.5 per cent, on the 200 gm. dose. Accordingly, the heat increment per 50 gm. of casein ingested at the different levels of feeding, from the lowest to the highest, was 19 calories, 27 calories, 39 calories, and 43 calories. When these data are plotted, a curve of heat production is obtained which is very similar to the curve of heat production (Fig. 1) derived at this Institute.

Williams, Riche and Lusk (4) recalculated the values given by Gigon above, by relating the increase in heat production not to the increase in protein ingested but to the increase in protein metabolism, and obtained the following heat-increment values per 100 calories of protein katabolized at the different levels of feeding:

50 gm. casein	.....	67	cals.
100 " "	.....	56	"
150 " "	.....	83	"
200 " "	.....	74	"
Average	.....	70	"

These values do not show a progressive increase with rise in plane of nutrition, as is shown by Gigon's original heat-increment values, which indicates that the katabolism of body protein below maintenance includes a factor of specific dynamic action (or waste heat of utilization). This factor may account, to a large extent, for the relatively low heat-increment values of the protein at the low planes of nutrition as obtained by relating the heat production to the food ingested.

In either case, however, it is apparent that

the specific dynamic action of protein is not a constant, but that it differs with the plane of nutrition at which it is determined—which is in harmony with the foregoing interpretation of the curve of heat production.

However this curve of heat production is explained, and whatever the ultimate causes of the contributing phenomena, it may be considered as the resultant of the combination of individual curves representing the metabolism of protein, carbohydrate, and fat; and it is the purpose of the present paper to analyze the curve of heat production by resolving it into these components.

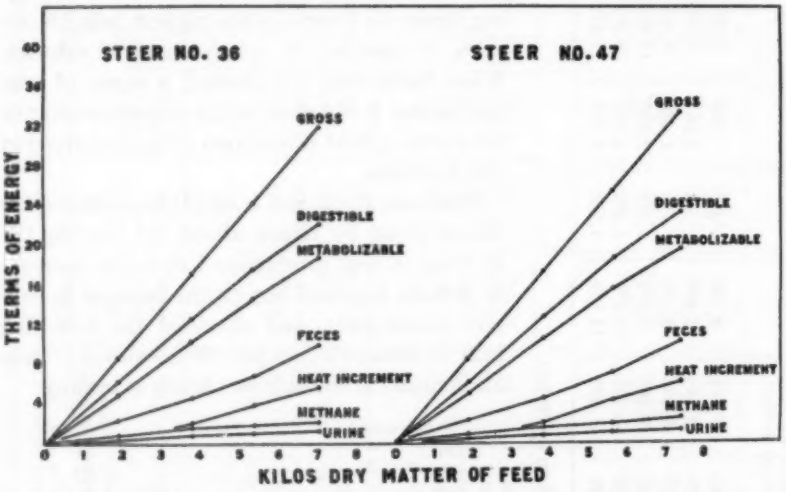

FIG. 3. The partition of the energy of the feed of cattle at different planes of nutrition.

PARTITION OF THE ENERGY OF THE FEED AND THE HEAT PRODUCTION OF CATTLE AT DIFFERENT PLANES OF NUTRITION

Figures 3 and 4 represent the partition of the gross feed energy, at the several planes of nutrition, between energy of digestible and of metabolizable nutrients, energy of urine, feces and methane, and energy expense of feed utilization—that is, heat increment.

In the experiments under discussion, no determinations were made of the oxygen consumption. Consequently no respiratory quotients were available for the determination of the proportions of the protein, carbohydrate and fat katabolized. It was found possible, however, to compute the energy of katabolism of these nutrients, as well as the energy of transformation of carbohydrate into fat, from the values determined for the production of

heat, urinary nitrogen, carbon dioxide, and methane. This computation, the results of which are presented in Table II, was accomplished as follows:

The energy and carbon dioxide resulting from the protein katabolized were computed from the urinary nitrogen by the use of Loewy's (5) factors (26.51 cals. and 4.75 liters CO_2, per gram of nitrogen). These quotas subtracted from the total heat production and total CO_2 production, respectively, leave the non-protein energy and non-protein CO_2.

The CO_2 of fat katabolism was then computed by means of the equation (*liters non-protein* CO_2 + *liters* CO_2 *from* $CH_4 - x$) $5.047 + 6.628x =$ *non-protein Calories* + *Calories from* CH_4, in which x represents liters of CO_2 from fat katabolism; the figure 5.047 represents the calorific equivalent of a liter of CO_2 from carbohydrate oxidation; and the figure 6.628 represents the calorific equivalent of a liter of CO_2 from fat oxidation.

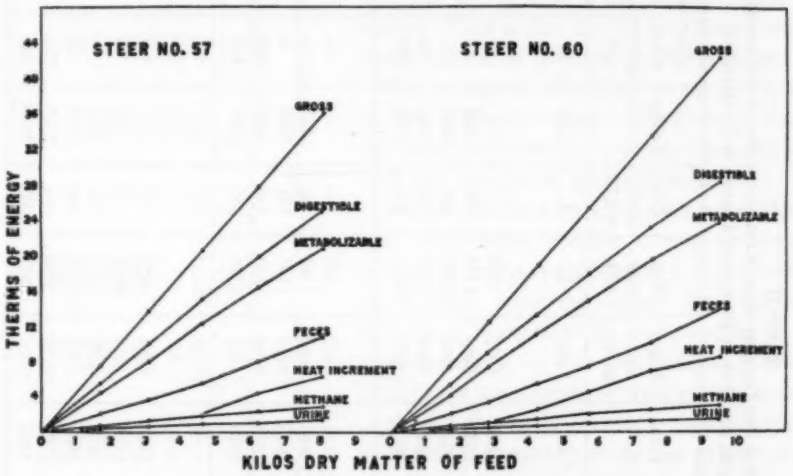

FIG. 4. The partition of the energy of the feed of cattle at different planes of nutrition.

The addition of the CO_2 from the oxidation of CH_4 to the non-protein CO_2, and of the energy equivalent of the CH_4 to the non-protein energy, is based on the principle evolved by Andersen (6) which places the metabolism of carbohydrate in a ruminant on a par with that in other species in which the carbohydrate katabolism is complete.

The calories from fat katabolism were then obtained by multiplying the liters of CO_2 from fat oxidation by 6.628.

The energy of carbohydrate katabolism in the submaintenance periods is the difference between non-protein energy and energy from fat oxidation.

TABLE II

COMPUTATION OF THE ENERGY OF PROTEIN, FAT AND CARBOHYDRATE KATABOLISM AND OF THE ENERGY OF TRANSFORMATION OF CARBOHYDRATES INTO FAT AT DIFFERENT PLANES OF NUTRITION

Experiment, animal and period No.	Urinary N	Prot. energy N×26.51	Prot. CO_2 N×4.75	Total heat prod. uncor. to stand. day	Total CO_2	Non-prot. energy	Non-prot. CO_2	CO_2 equiv. to CH_4	Energy of CH_4	CO_2 of fat synthesis*	Energy of fat synthesis	CO_2 of fat katab.†	Energy of fat katab.	Energy of carboh. katab.
	Grams	Cals.	L.	Cals.	L.	Cals.	L.	L.	Cals.	L.	Cals.	L.	Cals.	Cals.
Exp. 238														
Steer 36														
(Fasting) 12	42.2	1119	200	7651	1188	6532	988	—	—	—	—	978	6482	50
6	41.9	1111	199	8156	1531	7045	1332	87	831	—	—	452	2996	4049
8	47.1	1249	224	9840	2041	8591	1817	154	1481	—	—	79	524	8067
4	70.9	1880	337	11854	2549	9974	2212	192	1842	80	87	—	—	9887
2	83.4	2211	396	13888	3013	11677	2617	226	2168	127	138	—	—	11539
Steer 47														
(Fasting) 11	37.4	991	178	7396	1146	6405	968	—	—	—	—	961	6370	35
5	41.3	1095	196	7755	1463	6660	1267	86	827	—	—	416	2757	3903
7	47.9	1270	228	9383	1975	8113	1747	151	1449	4	4	—	—	8109
3	74.2	1967	352	11693	2572	9726	2220	196	1880	149	162	—	—	9564
1	87.1	2309	414	13536	3010	11227	2596	255	2445	181	197	—	—	11030
Exp. 240														
Steer 57														
(Fasting) 14	39.2	1039	186	7482	1158	6443	972	—	—	—	—	972	6442	—
10	39.7	1052	189	7939	1456	6914	1267	78	745	—	—	551	3652	3262
2	47.7	1265	227	7909	1652	6644	1425	133	1272	—	—	34	225	6419
4	44.9	1190	213	9493	2089	8303	1876	186	1781	82	89	—	—	8214
6	60.3	1599	286	11857	2702	10258	2416	244	2335	210	229	—	—	10029
8	79.3	2101	377	14408	3294	12306	2917	307	2930	262	286	—	—	12020

Steer 60 (Fasting)	15	43.2	1145	205	6904	1074	5759	869	—	—	—	869	5759	—
	11	39.1	1037	186	7476	1384	6439	1198	77	738	—	469	3109	3330
	1	40.4	1071	192	7253	1493	6182	1301	111	1061	—	74	490	5692
	3	39.8	1055	189	8821	1909	7766	1720	155	1483	54	59	—	7707
	5	49.7	1318	236	11157	2474	9839	2238	213	2086	112	122	—	9717
	7	66.5	1763	316	13976	3147	12213	2831	264	2518	225	245	—	11968
	9	88.4	2343	420	16133	3714	13790	3294	322	3075	350	382	—	13408

* Let x = liters CO_2 resulting from the conversion of carbohydrates into fat; then (non-protein CO_2 + CO_2 from $CH_4 - x$) $5.047 + 1.09x$ = non-protein energy + energy of CH_4. Solve for x.

† Let x = liters CO_2 of fat katabolism; then (non-protein CO_2 + CO_2 from $CH_4 - x$) $5.047 + 6.628x$ = non-protein energy + energy from CH_4. Solve for x.

The energy of transformation of carbohydrates into fat was computed by the use of the equation (*non-protein* CO_2 + CO_2 *from* $CH_4 - x$) $5.047 + 1.09\,x$ = *non-protein energy + energy of* CH_4, in which χ, represents liters of CO_2 resulting from the conversion of carbohydrates into fat; the figure 5.047 is the calorific equivalent per liter of CO_2 from carbohydrate oxidation; and the figure 1.09 is the calorific equivalent (7) of a liter of CO_2 resulting from the synthesis of fat from carbohydrates.

The energy of carbohydrate katabolism in the supermaintenance periods is the difference between the non-protein energy and the energy of fat synthesis.

These calculations are based upon the well established factors commonly employed in indirect calorimetry. Adequate discussions may be found in Lusk's Science of Nutrition (7), and in other reference works, of the derivation of the factors for computing protein energy and protein CO_2; of the caloric equivalents of the CO_2 resulting from the oxidation of carbohydrates and fat; and of the heat liberated in the conversion of carbohydrates into fat.

The method of indirect calorimetry, as it applies to cattle, including the principle of Andersen for accounting for the methane fermentation, has been critically discussed by Forbes and associates (8).

The whole process of resolving the heat production into its components, which has been employed in this paper, is in reality only the reverse of the process of computing the total heat production by indirect calorimetry, as adapted by Andersen on account of the extensive production of methane in the ruminant alimentary tract.

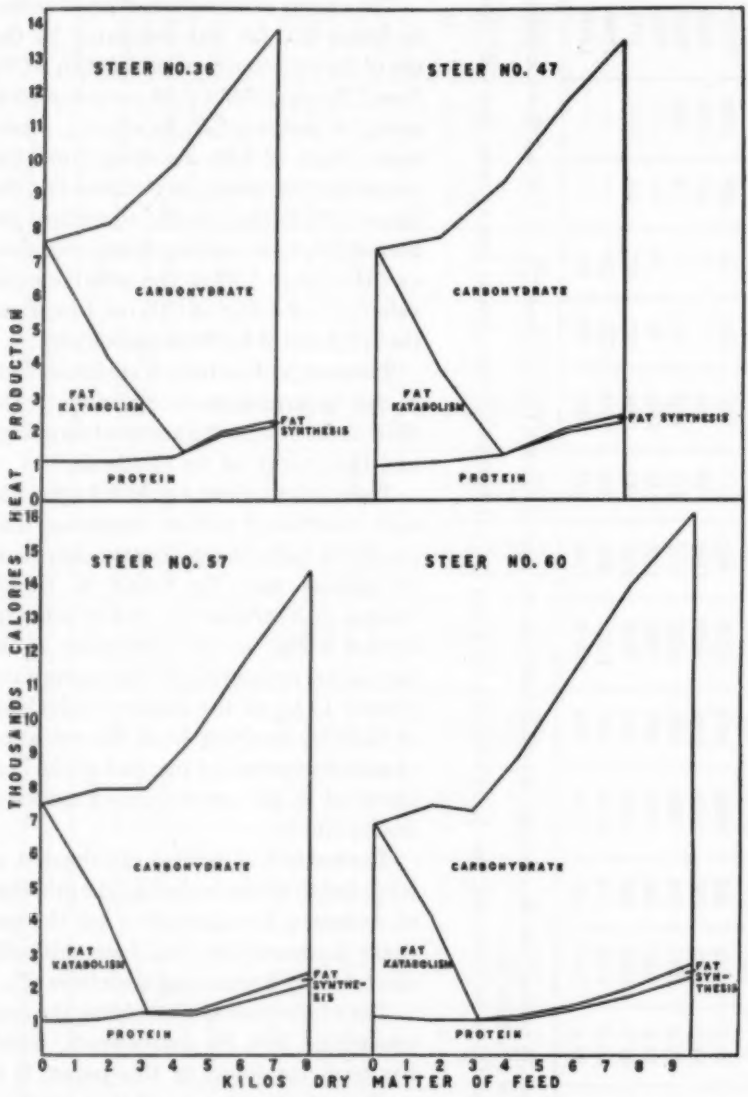

FIG. 5. The partition of the heat production of four steers at planes of nutrition between fast and two to three times the maintenance requirement of energy.

The elements of error in the factoring of the heat production, therefore, are exactly the same as obtain in the computation of the heat production by Andersen's modification of the standard respiratory quotient procedure.

As to the element of error in the starting point of this computation, that is, the directly determined heat production, Forbes and associates (8) compared 18 such measurements of the heat production of cattle with parallel computations of the heat production by the respiratory quotient method, as adapted by Andersen, and found that all of the computed values were between 98 and 104.8 per cent of the directly observed values, the average being 102.1 per cent, with a standard deviation of 2.15 ± 0.25 per cent.

Attempts have been made to derive further confirmation of the findings here reported, from the extensive calorimetric data on record at this Institute. A study of these materials shows unmistakably that they conform to the principles herein set forth, but they are not cited in detail since they are less perfectly adapted to the demonstration of these matters than are the results upon which this paper is based.

The contributions to the total heat production of heat from protein, from fat, and from carbohydrate, at the different planes of nutrition, are presented in Table II and in Figure 5, the separate quotas being parts of the directly observed heat production without reference either to the differences in the live weight of the animals, in the several experimental periods, or to the differences in the intervals of time spent by the animals in the standing and the lying positions.

In this relation it is significant that the sequence of the experimental periods (as already explained) was purposely arranged otherwise than in natural serial order, which determined the fact that the live weights, and therefore the maintenance quotas, also differed otherwise than in natural serial order. On this account the curves of uncorrected heat production in Fig. 5 are not so nearly symmetrical as are the curves in Figs. 1 and 2, which are based on the heat production corrected to represent uniform live weight and maintenance requirement.

The same fundamental data as referred to in the foregoing paragraph, computed to percentages of the total heat production, are presented in Table III, and in Figs. 6 and 7.

The energy of protein katabolism with the four animals constitutes a nearly constant proportion of the total heat production.

The values for energy of fat katabolized during periods of fasting (Table II, 13th column of data) are approximately the same as the values for non-

protein energy (6th column of data), in experiment No. 238, and exactly the same in experiment No. 240.

This signifies that in these periods the animals were in a state of true fast, with no oxidation of carbohydrate. The fasting heat measurement with steer No. 36 was during the fifth, sixth, and seventh days; with No. 47 during the fourth, fifth and sixth days; and with Nos. 57 and 60 during the fourth day, of fast—thus signifying that the equations and factors used in the computation are satisfactory.

TABLE III

PERCENTAGE CONTRIBUTIONS OF HEAT FROM PROTEIN, FAT AND CARBOHYDRATES TO THE TOTAL HEAT PRODUCTION AT DIFFERENT PLANES OF NUTRITION

Experiment, animal and period No.	Plane of nutrition	Energy of protein katab.	Energy of carbohydrate katab.	Energy of fat katab.	Energy of fat synthesis
Exp. 238					
Steer 36					
Period 12	Fasting	14.6	0.7	84.7	—
" 6	½ maintenance	13.6	49.6	36.7	—
" 8	Maintenance	12.7	82.0	5.3	—
" 4	1½ times maintenance	15.9	83.4	—	0.7
" 2	2 times maintenance	15.9	83.1	—	1.0
Steer 47					
Period 11	Fasting	13.4	0.5	86.1	—
" 5	½ maintenance	14.1	50.3	35.6	—
" 7	Maintenance	13.5	83.4	—	—
" 3	1½ times maintenance	16.8	81.8	—	1.3
" 1	2 times maintenance	17.1	81.5	—	1.3
Exp. 240					
Steer 57					
Period 14	Fasting	13.9	—	86.1	—
" 10	½ maintenance	13.3	40.7	46.0	—
" 2	Maintenance	16.0	81.2	2.8	—
" 4	1½ times maintenance	12.5	86.6	—	0.9
" 6	2 times maintenance	16.9	81.2	—	1.9
" 8	2½ times maintenance	14.6	83.4	—	2.0
Steer 60					
Period 15	Fasting	16.6	—	83.4	—
" 11	½ maintenance	13.9	44.5	41.6	—
" 1	Maintenance	14.8	78.4	6.8	—
" 3	1½ times maintenance	12.0	87.3	—	0.7
" 5	2 times maintenance	11.8	87.1	—	1.1
" 7	2½ times maintenance	12.6	85.6	—	1.8
" 9	3 times maintenance	14.5	82.2	—	2.4

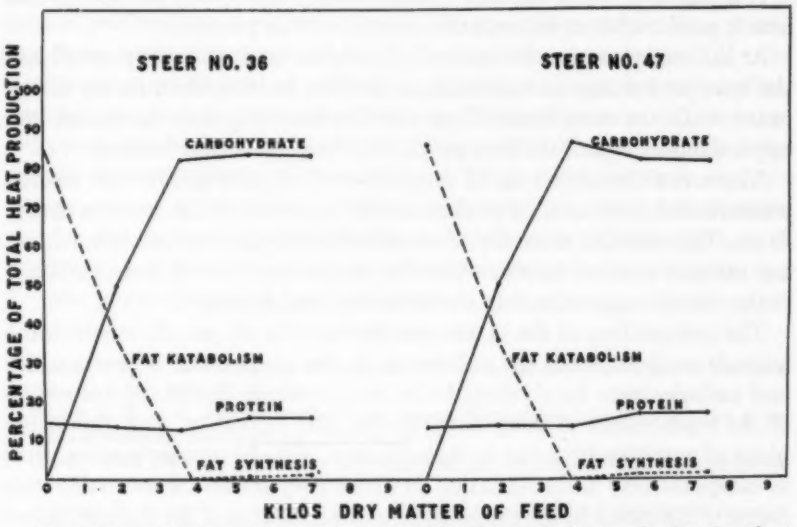

FIG. 6. The contributions of protein, fat, and carbohydrate to the heat production of cattle at different planes of nutrition.

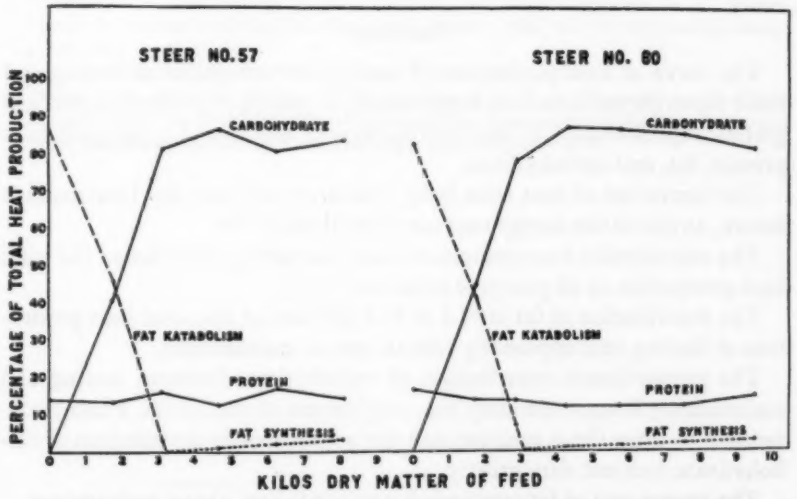

FIG. 7. The contributions of protein, fat, and carbohydrate to the heat production of cattle at different planes of nutrition.

It is shown in Table III that during fasting, 83.4 to 86.1 per cent of the heat is produced from fat, and the remainder from protein.

At half-maintenance, fat and carbohydrate contribute nearly equally to the heat production; at maintenance the heat production from fat disappears, while the contribution from carbohydrate is greatly increased, and approximately equals the heat production from fat during fasting.

Above maintenance a small proportion of the heat production results, without oxidation, as a by-product of the synthesis of fat from carbohydrate. This fraction naturally increases with rise in the plane of nutrition, but remains a minor factor; while the proportion derived from carbohydrate remains approximately the same as at maintenance.

The composition of the ration was the same in all periods in which the animals received food; the differences in the proportions of protein, fat and carbohydrate katabolized below maintenance, therefore, are results of the replacement of body nutrients by food nutrients, with rise in the plane of nutrition from fast to maintenance; and above maintenance there is comparatively little variation in these proportions. Presumably this factor of difference in the proportions of the nutrients of the different kinds which are metabolized at the different planes of nutrition contributes in some degree to the curve of heat production in relation to food consumption, but exact information as to the extent of this component is lacking.

Summary

The curve of heat production of cattle, between points of fasting and three times the maintenance requirement, is analyzed by computation and graphic representation of the contributions of heat from the katabolism of protein, fat, and carbohydrate.

The derivation of heat from body substance and from food nutrients is shown, as also is the energy expense of synthesis of fat.

The contribution from protein is about the same proportion of the total heat production at all planes of nutrition.

The contribution of fat is 83.4 to 86.1 per cent of the total heat production at fasting, and apparently falls to zero at maintenance.

The proportionate contribution of carbohydrate between fasting and maintenance is approximately the complement of that of fat. From maintenance to three times maintenance the proportionate contribution of carbohydrate does not vary greatly.

The energy cost of fat synthesis is a minor factor, above maintenance.

The causes of the curvature of the line representing the relation of the heat production to the food consumption of cattle, with rise in the plane

of nutrition from fasting to full feed, seem to be virtually as suggested in former publications, 1.—the increase in intermediary metabolism resulting from increasing concentration of circulating metabolites, 2.—the decrement of waste heat of utilization of body nutrients, between fasting and maintenance, 3.—changes in the proportions of protein, fat, and carbohydrate katabolized, and probable (but unestablished) differences in the specific dynamic effects of these different katabolized nutrients, 4.—the energy expense of synthesis of fat from carbohydrate, above maintenance, and 5.—the decreasing metabolizability of the food at the higher planes of nutrition, 6.—the heat of fermentation of carbohydrate nutriment, and 7.—the physical work of food utilization.

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IS THE VITAMIN B CONTENT OF MILK UNDER PHYSIOLOGICAL CONTROL?*

By

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INVESTIGATIONS bearing on the vitamin B content of milk may be said to have started with the work of Hopkins when he found in 1906 and 1907 that 2 cc. of milk sufficed for a young rat's need for accessory food factors. These results were published in 1912 by Hopkins (1). Osborne and Mendel (2) in 1911 reported success with rats which were used in determining the comparative nutritive value of various proteins when their synthetic diets contained protein-free milk. Later (3) they reported "a considerable degree of success in the absence of milk protein as well as of the hypothetical organic 'hormones', etc., present in the milk." Hopkins and Neville (4) were not so optimistic after their feeding trials. They demonstrated failure of growth unless their synthetic rations were supplemented with 2 cc. of milk per diem. This amount produced practically normal growth. Osborne and Mendel were able, after continuing their experiments over a longer period of time (5), to substantiate these results except with respect to the amount of milk required. A supplement of 2 cc. of milk daily rarely sufficed to allow the animals to make more then a very slight gain in weight. They state (p. 542) "It scarcely seems plausible that milk from different sources, even though produced from cows on unlike feeds, should account for the wide variation noted; in fact, we have used samples from several local sources, always with the same result," and (p. 541) "Not until at least 16 cc. of fresh milk per day were supplied along with the food mixture was anything approaching a normal rate of growth secured." In 1920 Osborne and Mendel (6) duplicated the ash of Hopkins' synthetic ration as closely as they could and obtained the same results as before. Even 2 cc. of unpasteurized milk when produced by cows on pasture supported but little, if any, growth in the young rats. Thereupon, Hopkins (7) reported further experiments in an attempt to clear up the discrepancies, anticipating possible differences in the nutritive value of milk or the response of the rats with change of season. Some of these experiments were carried out with goat's milk and others with cow's milk. Some of his re-

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sults obtained during the winter were, as stated by him, "frankly disappointing," but the favorable effect of 2 cc. of milk was nevertheless unmistakable and all of his other experiments proved as stated by him (p. 723) "that given the right conditions my original observations can be repeated." Stammers (8) obtained results, which, while they did not disprove Osborne and Mendel's conclusions, threw additional weight of evidence in favor of Hopkins' findings. Coward and Clark (9) obtained normal growth on 8 cc. Larger or smaller amounts were apparently not fed. These findings taken all together leave the situation unexplained.

In 1920, impressed by the necessity for clarifying the situation, we initiated a series of experiments in which we tested the influence respectively of breed, pasture, early lactation, late lactation, and the effect of increasing the vitamin B[1] content of the ration. The results of these experiments are shown in Chart 1.

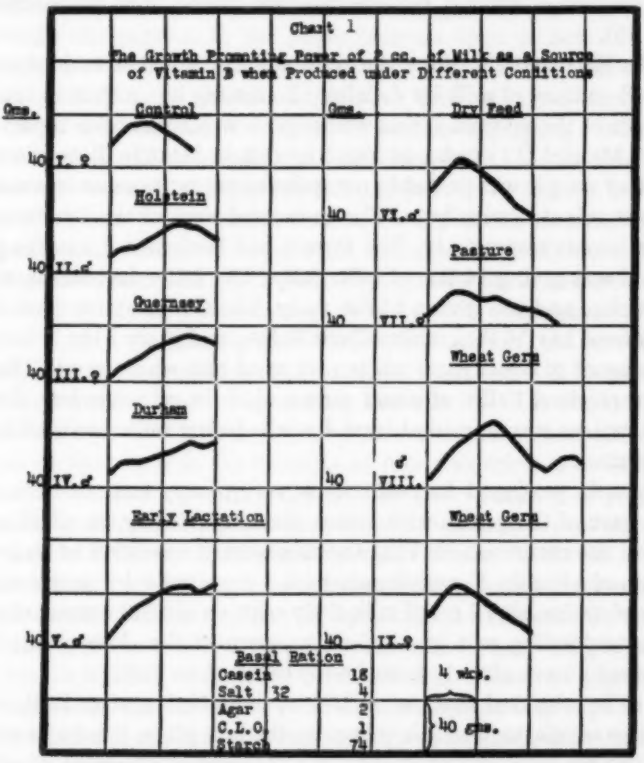
The rats used for these feeding trials were males and females weighing approximately 40 to 60 gms. at 23 to 27 days of age. They were kept individually on wood shavings in wire cages measuring 12×24×20 inches. Water and basal ration were fed *ad libitum*. The milk was pipetted off in small glass dishes. The basal ration consisted of; alcohol-extracted and heated casein 18 per cent, salts 32 (10) 4 per cent, agar 2 per cent, cod liver oil 1 per cent, and alcohol-extracted, cooked starch 74 per cent.

The data shown in Chart 1 present the results obtained with only one rat out of the 4 in each group selected for its representative performance. Graph 1 shows the growth obtained on the basal ration; the others show the result of supplementing the basal ration with 2 cc. of milk obtained from the various sources.

Graphs II, III, and IV, were obtained with milk produced by cows of the Holstein, Guernsey, and Durham breeds respectively. We considered it worth while to test out different breeds because of the possibility that Hopkins might have obtained his data from cows of the Durham breed. The cows used by us received a grain mixture composed of; yellow maize 3 parts, oats 3 parts, bran 3 parts, oil meal 1 part, and cotton seed meal 1 part, together with hay and silage. The Holstein cow had been milked continuously for 15 months and at the time of the experiment was producing 9 lbs. of milk daily. The Guernsey cow had been milked for 8 months and was producing 8 lbs. daily. Exact data on the Durham cow could not be obtained. She was suckling a calf on another experimental project and was producing barely enough for its needs.

[1] In this paper the term vitamin B refers to the ensemble of factors formerly thought of as a single entity.

Graph V shows results obtained with milk from a recently freshened Holstein cow during the months of October and November. She was giving 27 lbs. of milk daily when the experiment was started one week after parturition. It is evident from comparison with Graph II, which was obtained with milk from the Holstein cow, 15 months after parturition, that the stage of lactation was no factor in vitamin B secretion. Furthermore,



these graphs in comparison with Graphs III and IV show that the vitamin B content, at least under the conditions of our experiment, was not subject to variations with breed.

Graphs VI and VII were obtained with milk from a cow which was kept on dry feed during the months of May and June and then changed to pasture during July and August. She was of the Holstein breed and was producing about 40 lbs. of milk daily. During the dry feed period, she received daily 12 lbs. of alfalfa hay, 30 lbs. of corn silage and 12 lbs. of a

grain mixture which contained constituents mixed in the proportion; maize 4 lbs., oats 8 lbs., and oil meal 3 lbs. During the pasture period, she received 18 lbs. of the grain mixture and fresh alfalfa in her stall during the daytime and mixed pasturage at night. The rat experiments were started 6 weeks after parturition. From the results obtained, already referred to by Hart (11), it is evident that fresh succulent feed had no effect on the secretion of vitamin B. This confirms observations of Osborne and Mendel (6).

Graphs VIII and IX show the results of our attempt to increase the vitamin B content of milk by definitely increasing its amount in the feed. The source of the vitamin B was wheat germ which has been reported by Bell and Mendel (12) to be 10 times as rich in vitamin B as the wheat kernel. Our sample was probably not quite so potent because it was somewhat contaminated with bran. The cows used were of the Guernsey and Holstein breeds respectively. The former had freshened 4 months previously and was giving 14 lbs. of milk daily. The latter had freshened one month before and was giving 12 lbs. daily. The Guernsey received a mixture of mixed hay 10 lbs., maize silage 30 lbs., and grain 7 lbs. This latter was composed of equal parts whole corn meal and wheat germ. The Holstein cow received 10 lbs. of wheat germ and 10 lbs. of clover hay. Feeding of these rations was started at least 4 weeks before milk was used for the experiments.

The graphs produced herewith show very clearly that in spite of replacing part of the grains with wheat germ, absolutely no effect on the growth of the rats resulted. That the rats were in condition to respond to additions of vitamin B was demonstrated repeatedly by supplementing their basal ration plus 2 cc. of milk daily with an alcohol extract of wheat germ corresponding to 6 gms. of wheat germ at the close of the trials. These results have already been briefly referred to (13).

It is to be admitted that our technic of determining vitamin B in 1921 was not so satisfactory as it is today. In the first place, it is to be remembered that we were always dealing with the entire ensemble of vitamin B's and, furthermore, it was not until 1923 that we (14) pointed out the necessity of keeping rats on screens to reduce coprophagy to a minimum. As already stated, the rats in the preceding experiments had been kept on shavings. McCollum, Simmonds, and Becker (15) spoke disparagingly of the innovation of keeping rats on screens, pointing out that with frequent cleaning of cages, it is not necessary. They apparently overlooked the fact that two years before their paper was published, we had reported cleaning our cages daily, but this we found to be less effective and much

more laborious. At present, the use of screens is generally accepted as good technic in feeding experiments.

It is, however, scarcely probable that Hopkins obtained his pronounced growth effects in consequence of coprophagy. As a matter of fact, we are led to believe that he practically eliminated this factor because he states (1) that he used wire cages on legs 4 inches high to prevent consumption of filter paper placed below and that the greater part of the feces fell through the cage on to the paper beneath. One gathers the impression that his screen bottoms were probably of rather smaller mesh than those used by us. He, furthermore, emphasizes the necessity of cleanliness.

There are, however, other factors besides coprophagy which demand consideration. In analyzing data obtained with rats on low vitamin B diets, one occasionally notices unusual growth in certain individuals or groups. These performances cannot be explained on the basis of pre-experimental storage of vitamin B, because it is well known that such storage does not take place (16). These, likewise, are scarcely to be explained by difference in consumption of excreta, although certain individuals and correspondingly, therefore, certain groups, are sometimes unusually adept at coprophagy. Scheunert and Schieblich (17) found that vitamin B was synthesized by certain bacteria found in the intestine. Heller, McElroy, and Garlock (18) produced evidence of bacteriological synthesis of vitamin B in the intestine of rats. Fridericia *et al.* (19) provided actual evidence for possible dispensability of vitamin B in the ration by what they termed refection. This they attribute to a probable synthesis of vitamin B by certain organisms which develop in the intestinal tract under certain experimental conditions. Roscoe (20) observed the phenomenon of refection independently at about the same time. Kon and Watchorn (21) confirmed these observations. Roscoe (20) reported that the rats in which it occurred were all piebald. Albino rats kept in the laboratory at the same time were not affected. Pierce, Osgood, and Polansky (22) reported a difference in the rapidity with which their strain of rats responded to lack of vitamin B in comparison with strains purchased from an animal dealer. However, they observed that their rats, which were piebald, were not so sensitive as the albino. Even though there is no unanimity of conclusions on resistance of different strains, the question may well be raised whether it is possible that Hopkins had refection in his milk fed animals but not in his controls.

That synthesis of vitamin B can occur through action of micro-organisms in the rumen of the cow, was suggested by Theiler, Green, and Viljoen (23) and has been demonstrated by Bechdel and co-workers (24,

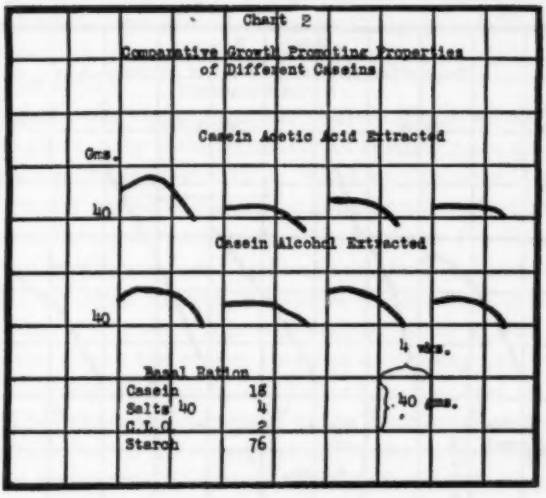
25, 26). The last named not only demonstrated that the amount synthesized was sufficient to maintain normal growth in young heifers, but also that it was sufficient to produce milk of a vitamin B content normal or only slightly less than normal. However, continued lactation was not accomplished,—disaster supervened after one or two weeks. It should be mentioned that such a synthesis of vitamin B may not always occur in the ruminant. Hughes, Fitch and Cave (27) for instance, have reported low vitamin B values in cow's milk produced on a low vitamin B ration. Kennedy and Dutcher (28) made similar observations.

Reference should also be made to the recent noteworthy findings of Evans and Lepkovsky (29) who demonstrated that an increase in the fat content of the diet decreased the rats' requirements for vitamin B. This they associated with the presence of certain fatty acids and mixtures of fatty acids in the glycerides. It is admitted that our rations were low in fat, carrying only 1 per cent of cod liver oil and the fat of the milk supplement. Hopkins (1) used a basal ration containing 12.4 per cent of lard, but Osborne and Mendel (5) used a basal ration which contained 18 per cent of lard and 9 per cent of butter fat. It is, therefore, scarcely possible that the difference in effectiveness of the milk supplements can be explained by difference in intake of fatty acids, even in full recognition of the variability in fatty acid content of fat produced under different conditions.

Within the last several years we have had occasion to reinvestigate the remarkable constancy in the vitamin B content of milk, as used in our laboratory, not to mention the discrepancy in our results compared with those of Hopkins. We carried out these new experiments with both cow's and goat's milk, preventing coprophagy by the use of screen cage bottoms and giving full recognition to the existence of other vitamins in the vitamin B complex. The cows' milk was obtained from the university herd consisting mostly of cows of the Holstein breed with some of the Jersey, Guernsey, Ayrshire, and Brown Swiss breeds as well. The goats were pure bred Toggenbergs or grades.

Male rats weighing approximately 60 gms., as raised in our laboratory, were used exclusively for the tests. They were fed a basal ration of casein 18 per cent, salt 40 (30) 4 per cent, cod liver oil 2 per cent, cooked starch 74 per cent. Sometimes 2 per cent of agar was included in this ration, but we were inclined to question the necessity for the use of roughage under the conditions of our experiment. Substances other than milk, when tested for vitamin B, were introduced into the ration at the expense of an equal amount of starch. Milk was fed as a supplement to the entire diet.

The casein used in our rat rations was Argentine acid casein, which had been purified by extraction with water acidified with acetic acid for a week. In some cases we used the same commercial casein which had been purified by repeated extraction with hot alcohol for a week. Preliminary feeding tests, the results of which are shown in Chart 2, showed that it was immaterial which casein was used. In both instances the amount of growth obtained compared favorably with that reported by Palmer and Kennedy (31) with casein purified by a much more laborious procedure.

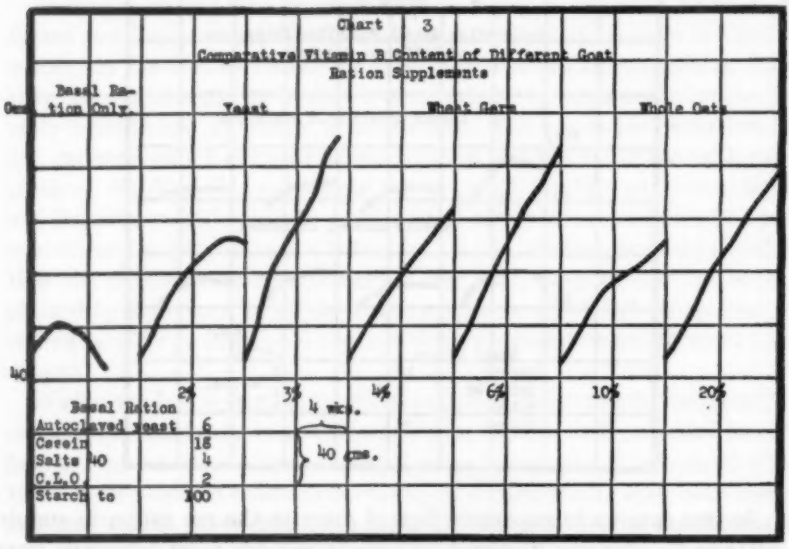


In one case we incorporated heated yeast in the rat ration to supply vitamin G. This was prepared by autoclaving dry yeast (obtained from the Northwestern Yeast Company) in a layer one inch thick in an enameled pan at 15 lbs. pressure for 2-1/2 hours, and then drying at 60 to 95° Centigrade. A test with four rats revealed the presence of only very small amounts of vitamin B when fed at a level of intake constituting 24 per cent of the ration. Three of the rats on this experiment died in from 5 to 7 weeks; on the basal ration alone, they died in from 5 to 6 weeks.

The goat rations were built up around a daily intake of 0.75 lb. of alfalfa and 1.5 lbs. of a grain mixture. This latter consisted of a basal mixture of 35 parts yellow corn meal, 30 parts wheat bran, 5 parts linseed oil meal, and 1 part of sodium chloride. This basal mixture was supplemented respectively with 30 parts of oats to make the "barn ration"; with 30 parts of wheat germ to make the "wheat germ ration," and with

15 parts each of yeast and oats to make the "yeast ration." Taking the daily ration, which amounted to 2.25 lbs. of hay and grain as a whole, the three rations differed from one another in that they contained respectively 20 per cent oats, 20 per cent wheat germ, and 10 per cent of dried yeast. We made numerous attempts to secure a larger percentage intake of wheat germ and yeast, but this tended to interfere with consumption.

In Chart 3, we present some results of tests designed to reveal the comparative vitamin B content of the oats, wheat germ, and yeast as used in our rations. In order to conserve space, only one rat of each group of four



was charted. Those charted were taken as being representative of the group. From their performance it appears that approximately 3 parts of yeast were equivalent in vitamin B content to 6 of wheat germ and 20 of oats. These amounts gave slightly better than normal growth, while 2, 4, and 10 parts, respectively, of the aforementioned constituents gave subnormal growth of approximately equal intensity.

In a previous publication we (14) have stated that approximately 60 parts of a grain such as oats are necessary for normal growth. However, in those cases the grain was relied upon to furnish both vitamins G and B. It is now well known that at least the oat kernel is preponderatingly low in the former with respect to the requirements of rats (32). We accordingly supplied vitamin G in the present vitamin B assays of yeast, wheat germ,

and oats, in the form of autoclaved yeast. We did not use "equalized consumption" technic in these experiments, although in general we are committed to this procedure (33). The probability of error due to its non-use was not large, as revealed by our consumption data. With 2, 4, and 10 per cent of yeast, wheat germ, and oats respectively in the rations, the amounts consumed were 7.9, 10.4, and 8.9 gms.; with 3, 6, and 20 per cent of these materials the consumption was 9.9, 9.4, and 8.9 gms. respectively.

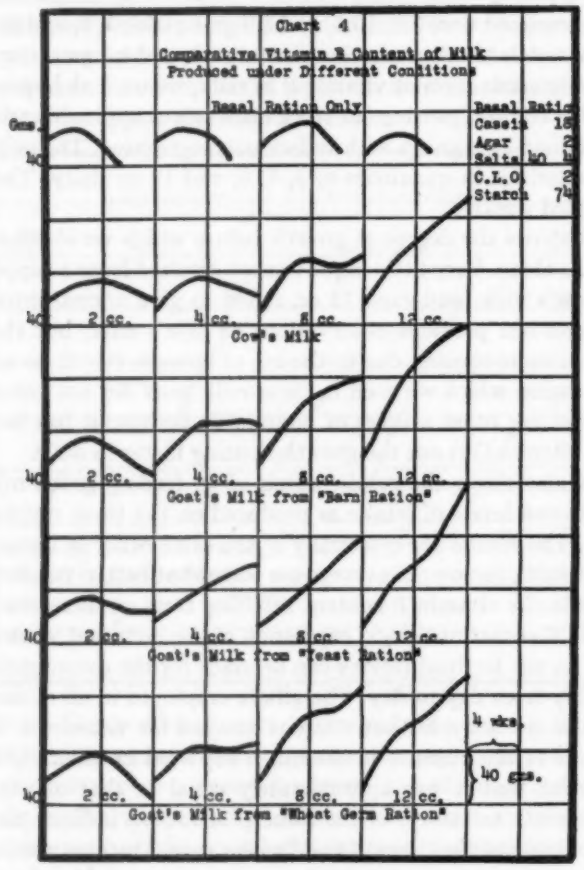
For the determination of vitamin B in milk, we used an improved form of our earlier technic, putting our stock male rats at approximately 60 gms. on screens, four in a group with individuals segregated. The milk was fed fresh, pipetted out in quantities of 2, 4, 8, and 12 cc. daily. The animals were weighed weekly.

Chart 4 shows the degree of growth failure which we obtained on our basal ration alone. Very little improvement resulted from a supplement of 2 cc. of cow's milk, and even 12 cc. failed to give normal growth. This substantiates our previous observations on cow's milk, but the present results were more regular, due to the use of screens. It will be noted that the basal ration which we used in these milk tests did not contain autoclaved yeast nor other sources of vitamin G, because it has been shown (36) that vitamin G is not the growth-limiting factor in milk.

Chart 4 also shows the results obtained on feeding goat's milk at the aforementioned levels of intake as produced on the three rations already described. The results are essentially of the same order as those obtained with cow's milk, but on the average are somewhat better. Again there was no change in the vitamin B content resulting from an increased intake in the ration. No exact numerical expression of the increased vitamin B consumption on the fortified rations can be made for the consumption varied considerably from day to day. The alfalfa employed in all of the goat rations was of the same lot but was not assayed for vitamin B. However, on the basis of the amount of vitamin B reported in alfalfa (34, 35), we may consider that it was approximately equal to that of whole cereal grains. Separate rat assays of the three goat rations indicate that the vitamin B content of the "yeast" and "wheat germ" rations was practically double that of the "barn ration." It seems safe to consider that the total vitamin B consumed by the goats was increased about two-thirds on each of the two fortified rations over that on the "barn ration."

Milk production of the goats varied from one to five pounds daily per individual. As a rule, the output approximated two pounds daily. Calculations to correlate the vitamin B intake of the goats with that secreted in the milk show that on the "barn ration" about fourteen per cent, and

on each of the fortified rations about nine per cent, of the consumed vitamin B occurred in the milk. In other words, the amount of vitamin B per unit of milk seems to remain unchanged when the dietary intake of vitamin B is stepped up from a reasonably good level to one about two-



thirds higher. Our experience with goat's milk also is definitely at variance with Hopkins' experience, because he (7) obtained nearly normal growth when he supplemented his basal ration with 5 cc. of goat's milk daily. We definitely failed to accomplish this result with 8 cc. but approached it with 12 cc.

Our failure to increase the vitamin B content of goat's and cow's milk

by unusual increases in the amount in the ration, stands in marked contrast to the observed effects of feeding rations markedly deficient in vitamin B. Andrews (37) in 1912 produced beriberi in 16 pups when these were nursed by women who had previously lost their infants from beriberi. Improvement in other infants was also observed with the substitution of less highly milled rice for the polished rice in the mother's diet. From these results it is apparent that the mother is less sensitive to impending deficiency than is the infant. In 1918 we encountered a similar situation in experiments with rats on a ration markedly low in vitamin B. We failed to notice any signs of impending disaster in the nursing mother, but observed that the young were seized with convulsions from which they failed to recover until they were put with a nursing female maintained on a normal ration (38, 39). In 1924, Sure (40) also observed that the vitamin B requirements of the rat are much greater for normal mammary function than for growth. Later, he (41) showed that they are at least 3 times as great and that the requirement of abnormally large amounts is due, at least in part, to the unusually large losses incurred in the mother's metabolism (42). Hartwell (43) reported that the lactating rat requires at least 4 times as much vitamin B as the growing animal. Evans and Burr (44) found that 5 times the usual intake of vitamin B in the form of yeast was required for normal nutrition of the young suckling rat. Macy, Outhouse, Graham, and Long (45), also found an increase in the vitamin B requirement of the rat during lactation. Daniels, Jordan, and Hutton (46) believe that the high mortality of nurslings of rats on milk is due not to low vitamin B intake, but rather to insufficient intake of calories. Sure (47) emphatically denied this.

In general, it may be said that there is no dearth of evidence to prove that the vitamin B content of milk can be reduced below normal, yet it must be remembered that the failure of experiments to produce growth in young rats may not always be due to poor quality of milk, but to a deficiency in quantity. Sure and Schilling (48), however, have reported poor growth in young rats with an abundance of curd in their stomachs. In the light of this, our observations that vitamin B cannot be increased above a certain amount, stand out as being all the more remarkable.

The sufficiency of the vitamin B content of milk, both human and cows', for normal nutrition of the infant has been widely discussed. Daniels and Byfield (49) obtained favorable results in infant feeding with the use of a wheat germ extract, but Daniels (50) was not so successful with brewer's yeast because it caused diarrhea as an apparent result of intestinal irritation. Bloxsom (51), however, advised the use of brewer's yeast as a sup-

plement to the diet of the infant from birth. Hoobler, Macy and Outhouse (52) believe that breast milk cannot be relied upon to supply the optimum amount of vitamin B for the infant. Hoobler (53) believes that every infant should have an addition of vitamin B to its milk diet, whether cow's or human. Dennett (54), in studying the value of a commercial preparation of vitamin B prepared from wheat germ, found it valuable for infant feeding with both cow's and mother's milk. It is, of course, entirely possible that in these instances the mother's milk was produced on a vitamin B-poor diet and, therefore, may have been abnormally low in vitamin B, but it is scarcely probable that the cow's milk was similarly impoverished.

In 1928 Macy, Outhouse, and Hunscher (55) pointed out that with their lactating human subjects the vitamin B content of milk varied approximately inversely to the production level. Later work from the same laboratories by McCosh, Macy, and Hunscher (56) confirms this opinion. These investigators found that supplementation of the maternal diet with 10 grams of yeast daily caused the occurrence in the milk of some additional factor which they concluded pointed to better utilization of the food of experimental animals. However, they add that "It is obvious that this substance is not an appetite stimulator; it is consequently improbable that it is the antineuritic vitamin." From their data we are unable to conclude whether or not the vitamin B content of the diet was optimal for the production of milk of normal vitamin B potency. However, it seems quite probable that the vitamin B level in the "pre-experimental" diets of the lactating human subjects of McCosh, Macy, and Hunscher was not particularly high. In a certain way, their general results with humans are analagous to our data on vitamin B as obtained with cows and goats.

Our findings that cow's milk and goat's milk cannot have their vitamin B content increased beyond the usual level emphasize the necessity for recognizing the definite limitation of cow's and goat's milk in normal human nutrition as claimed by others. It remains to be seen whether or not our findings and the findings of those who claim that cow's milk should be fortified with vitamin B for infant feeding will be supported by future investigations. Obviously, there are many possible variables which enter into the determination of such general relations, and in the face of everything Hopkins' outstanding conclusions need to be harmonized with those of others.

Conclusions

Hopkins' conclusions relative to the high concentration of vitamin B in cows' milk could not be confirmed. No essential differences were found

in the vitamin B content of milk produced by cows of the Holstein, Guernsey and Durham breeds. The period of lactation did not appear to have any influence. Milk produced on pasture and fresh green alfalfa did not contain any more vitamin B than milk produced on silage and dry feed, thus confirming results obtained by Osborne and Mendel. Increasing the vitamin B intake both absolutely and in relation to the amount of milk secreted had no discernible effect on the vitamin B content of cows' or goats' milk. It appears, therefore, that the maximum vitamin B content of milk is under definite physiological control.

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